Phytochemical analysis and green synthesis of gold nanoparticles from selected *Flacourtia* spp.

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I hereby declare that the data presented in this Dissertation / Internship report entitled **"Phytochemical Analysis and Green Synthesis of Gold Nanoparticles from Selected *Flacourtia* spp."** is based on the results of investigations carried out by me in the **Botany** at the School of Biological Sciences and Biotechnology, Goa University under the supervision of Dr. S. Krishnan and the same has not been submitted elsewhere for the award of a degree or diploma by me. Further, I understand that Goa University or its authorities will not be responsible for the correctness of observations / experimental or other findings given the dissertation.

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COMPLETION CERTIFICATE

This is to certify that the dissertation report **"Phytochemical Analysis and Green Synthesis of Gold Nanoparticles from Selected *Flacourtia* spp."** is a bonified work carried out by **Miss Veda Raju Dessai** under my supervision / mentorship in partial fulfilment of the requirements for the award of the degree of **M.Sc.** in the Discipline Botany at the School of Biological Sciences and Biotechnology, Goa University.

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(Miss Veda Raju Dessai)

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CHAPTER 1: INTRODUCTION

INTRODUCTION

Nanoscience has made breakthroughs in almost every field of science, and with this knowledge, the use of nanotechnologies makes life even more manageable in this evolving era (Kagan, 2016). Nanoscience can potentially reshape the world, creating a platform for rising nanoworld opportunities. Nanotechnology encompasses understanding the fundamental physics, chemistry, biology, and technology of nanometer-scale objects, thus making it a multidisciplinary field (Chan, 2016). Therefore, material scientists, mechanical and electronic engineers, and medical researchers are teaming up with biologists, physicists, and chemists. It has very well achieved the status as one of the most critical research endeavors of the early 21st century, as scientists harness the unique properties of atomic and molecular assemblages constructed at the nanometer scale (Scott and McNeil, 2005).

Research at the nanoscale is unified by the need to pool knowledge on tools and techniques and to share expertise. The advancement continues accelerating as researchers produce increasingly excellent new materials and products (Hornyak, 2018). The ability to manipulate these particles' physical, chemical, and biological properties enables researchers to rationally design and use nanoparticles for drug delivery, image contrast agents, and diagnostic purposes (Nouailhat, 2006). Development in fuel cells, vaccines, batteries, and construction materials, are all made possible through the successful implementation of nanotechnology. The rise in nanoworld work has gained recognition around the globe, and to celebrate the astounding growth and importance, the U.S. observed its first National Nanotechnology Day on October 9, since October is the tenth

month of the year, 10^{-9} pays homage to the nanometer scale 10^{-9} or a nanometer (Kagan, 2016).

1.1. The Origin of Nanotechnology

The first use of the concepts found in 'nanotechnology' was in 'There's Plenty of Room at the Bottom,' a talk was given on December 29, 1959, at an American Physical Society meeting at Caltech by the physicist Richard Feynman. Feynman described a process by which the ability to manipulate individual atoms and molecules might be developed, using one set of precise tools to build and operate another proportionally smaller group, and so on down to the needed scale. He received the Nobel Prize for this work and was regarded as the father of nanotechnology (Bayda *et al.,* 2019). During this, he noted, scaling issues would arise from the changing magnitude of various physical phenomena: gravity would become less important, surface tension and Van der Waals attraction would become increasingly more significant, etc. this basic idea appeared plausible and exponential assembly enhances it with parallelism to produce a valuable quantity of end products (Breck, 2016).

Tokyo Science University Professor Norio Taniguchi defined the term' nanotechnology' in a 1974 paper as 'Nanotechnology' mainly consists of the processing of separation, consolidation, and deformation of material by one atom or molecule' (Hulla, 2015). In the 1980s, the basic idea of this definition was explored in much more depth by Dr K. Eric Drexler, who promoted the technological significance of nanoscale phenomena and devices through speeches and the books Engines of Creation: The coming era of Nanotechnology and Nanosystems: molecular machinery, manufacturing, and computation and so the term acquired its current sense (Breck, 2016).

Nanotechnology and nanoscience started in the early 1980s with two significant developments; the birth of a cluster of science and the invention of the scanning tunneling microscope (STM). This development led to the discovery of fullerenes in 1985 and carbon nanotubes a few years later. The Buckminsterfullerenes $C_{60,}$ also known as the buckyball, is the simplest of the carbon structures known as fullerenes. In 2000, the United States National Nanotechnology Initiative was founded to coordinate Federal nanotechnology research and development (Breck, 2016).

1.2. Fundamentals of Nanoscience and Nanotechnology

Nanoscience is the study of structures and materials on an ultra-small scale and the unique and interesting properties materials demonstrate (Poole, 2003). It is cross-disciplinary, thus letting scientists from various fields, including chemistry, physics, biology, medicine, computing, materials science, and engineering, study and use it to understand the world better. While nanotechnology is the term given to those areas of science and design where peculiarities that happen at aspects in the nanometer scale are used in the planning, characterization, creation, and utilization of materials, constructions, gadgets, and frameworks (Omran, 2020).

"Nano" is a prefix denoting the minus ninth power of ten, namely one billionth. 1nm is a minimal length corresponding to one billionth of 1 m, one-millionth of 1 mm, or one-thousandth of 1mm (Naito *et al.*, 2018); nanosized particles exhibit entirely new or improved properties as compared to the larger particles of the bulk material with specific characteristics, such as size, distribution, and morphology (Willems, 2005). The reason for the intense interest of scientists nowadays in nanoparticles is that nanoparticles can exhibit different properties and functions than regular bulk materials. The most crucial factor that enables the production of nanostructures in the desired size, shape, and properties and

provides their usage in various fields is that the effects of classical physics are reduced, and quantum physics becomes active (Nadaroglu, 2017).

Nanoparticles can be found to be formed in many morphologies, from spheres through flakes and platelets to dendritic structures (Pitkethy, 2003). According to their origin; nanomaterials are classified into two main groups; natural nanomaterials found in nature such as viruses, proteins, enzymes and minerals, and artificial nanomaterials not found in nature require some processes for their production. According to their dimensions, nanomaterials are examined under four classes: nanosized nanocrystals -also known as zero dimensions, including metallic and semiconductor nanoparticles. One-dimensional nanomaterials include nanowires, nanobots, and nanotubes. Dimensional nanomaterials such as nanocomposites and nanoplates, and three-dimensional nanomaterials, bulkers. According to their structural configurations, nanomaterials are studied under four main groups metallic nanomaterials, carbon-based nanomaterials, dendrimers, and composites **(**Hornyak, 2018).

1.3. Synthesis of Nanoparticles

In synthesizing nanoparticles, which can be of natural or synthetic origin and exhibit unique properties at the nanoscale, two basic approaches that include various preparation methods and are known from early times are used. The first approach is the "top-down" method which calls for the breaking down solid materials into small pieces by applying external force. This approach uses many physical, chemical, and thermal techniques to provide the necessary energy for nanoparticle formation (Nadaroglu, 2017). The second approach, "bottom-up," is based on gathering and combining gas or liquid atoms or molecules. These two approaches have advantages and disadvantages relative to each other. In the up-down approach, which is costlier to implement, it is impossible to

obtain perfect surfaces and edges due to cavities and roughness that can occur in nanoparticles.

In contrast, excellent nanoparticle synthesis results can be obtained by the bottom-up approach (Arole and Munde 2014). In addition, with the bottom-up approach, no waste materials that need to be removed are formed, and nanoparticles having smaller sizes can be obtained because of the better control of the dimensions of the nanoparticles. The mechanical abrasion meth is listed under the top-down approach, which uses various ball mills to break down the material into particles producing nanosized alloys, composites, and semi-crystalline structures. Although this method is inexpensive, efficient, and straightforward, it is susceptible to contamination caused by the balls (Singh, 2004).

1.4. Approach towards Green Synthesis of Nanoparticles

The biological method, an alternative to chemical and physical processes, provides an environmentally friendly way of synthesizing nanoparticles. Moreover, this method does not require expensive, harmful, and toxic chemicals. Metallic nanoparticles with various shapes, sizes, contents, and physicochemical properties can be synthesized thanks to the biological method actively used in recent years. Synthesis can be done in one step using biological organisms such as bacteria, actinobacteria, yeasts, molds, algae, and plants or their products. Molecules in plants and microorganisms, such as proteins, enzymes, phenolic compounds, amines, alkaloids, and pigments, perform nanoparticle synthesis by reduction. In traditional chemical and physical methods, reducing agents involved in removing metal ions and stabilizing agents used to prevent undesired agglomeration of the produced nanoparticles carry a risk of toxicity to the environment and the cell. Besides, the produced nanoparticles' contents are considered toxic regarding shape, size, and surface chemistry (Nadaroglu, 2017).

In the green synthesis method in which nanoparticles with biocompatibility are produced, these agents are naturally present in the employed biological organisms. Plant-mediated synthesis of nanoparticles is a green chemistry approach that connects nanotechnology with plants. The biosynthesis of nanoparticles has been proposed as a cost-effective and environmentally friendly alternative to chemical and physical methods. Novel methods of ideally synthesizing NPs are thus formed at ambient temperatures, neutral pH, low costs, and environmentally friendly fashion (Parveen *et al*., 2016). Plants, which have great potential for detoxification, reduction, and accumulation of metals, are promising, fast, and economical in removing metal-borne pollutants.

1.5. Metal Nanoparticles: with particular emphasis on AuNP's

An important aspect of nanotechnology that has a big challenge is the development of metal nanoparticle synthesis. Metallic nanoparticles having various morphological characteristics can be produced intracellularly and extracellularly. The synthesis process is initiated by adding extracts obtained from plant parts such as leaves, roots, and fruits into the aqueous solution of metal ions. The materials present in the plant extract, such as sugar, flavonoid, protein, enzyme, polymer, and organic acid, acting as a reducing agent, takes charge of the bio-induction of metal ions into nanoparticles (Shankar *et al*., 2004). Nanoparticles of noble metals, such as gold, silver, and platinum, are widely applied in products that directly encounter the human body, such as shampoos, detergent, soaps, shoes, cosmetic products, and toothpaste, besides medical and pharmaceutical applications (Shobha *et al*., 2017).

Gold has a long history of use. Chemical and physical methods were traditionally applied for the synthesis of gold nanoparticles. However, their use is accompanied by several drawbacks (Turkevich *et al*., 1951). The main disadvantages of the chemical

methods are to use of highly toxic reagents, environmental pollution, and carcinogenic solvents, and on the other hand, physical processes require expensive equipment and high energy consumption (Okamoto *et al*., 2019). In addition, the low stability of AuNPs, difficulties in controlling crystal growth, and particle aggregation make the above methods less advantageous.

The "green" method of AuNP synthesis is attracting more attention due to the expansion of nanotechnology capabilities. The critical factors of biological synthesis popularity are the use of non-toxic agents without additional stabilizers and reducing agents, renewable materials, low energy expenditure, and ecological safety. The living organisms' great diversity allows the production of specific, practice-oriented gold nanoparticles (Rawat *et al*., 2016). Moreover, the biomolecules involved in the biosynthesis by bacteria, fungi, algae, and plants positively affect the synthesis process.

1.6. Au NP's biosynthesis mechanism

The production of gold nanoparticles is a sufficiently simple process that does not require an increase in temperature and pressure. The general scheme assumes the following: the biological extract (bacterial, fungal, plant, etc.) is added dropwise to the $HAuCl_4$ salt solution and mixed well to initiate the AuNPs synthesis process (Gu *et al.*, 2021). The color change of the resulting solution indicates nanoparticle production. Although many publications illustrate AuNP synthesis using different organisms (bacteria, algae, fungi, plants), the mechanism of the biogenic process is not fully understood. The biosynthesis takes place in two steps: first, Au^{3+} is reduced to Au^0, and second, agglomeration and stabilization result in the AuNP formation (Sheny *et al.*, 2011). Interestingly, a wide variety of bio-compounds (enzymes, phenols, sugars, etc.) can participate in the gold reduction, stabilization, and capping of nanoparticles.

Red colloidal gold has been used as medicine for revitalization in China and India (Bhattacharya and Mukherjee 2008). Gold nanoparticles have found use in diagnostic and drug delivery applications (Bhumkar *et al.*, 2007). Gardea-Torresdey *et al*. (2002, 2003) demonstrated gold and silver nanoparticle synthesis within live alfalfa plants from solid media. Biosynthesis of Au nanoparticles by plants, such as *Azadirachta indica* (Shankar *et al*., 2004), *Medicago sativa* (Gardea-Torresdey *et al.,* 2002), *Aloe vera* (Chandran *et al*., 2006), *Cinnamomum camphora* (Huang *et al*., 2007), *Pelargonium graveolens* (Shankar *et al*., 2003), *Cymbopogon flexuosus* (Shankar *et al*., 2004), *Tamarindus indica* (Ankamwar *et al.,* 2005), *Coriandrum sativum* (Narayanan and Sakthivel 2008) have also been reported. Thus, extracellular nanoparticle synthesis using plant leaf extracts rather than whole plants would be more economical owing to easier downstream processing.

1.7. Botanical Description of the selected plant species

1.7.1. Genus *Flacourtia*

Flacourtia is a genus of flowering plants in the family Salicaceae. It was previously placed in the now-defunct family Flacourtiaceae—the generic name honors Étienne de Flacourt (1607–1660), a governor of Madagascar. Trees or shrubs, dioecious or hermaphroditic, rarely polygamous, usually spiny. Leaves alternate, petiolate; stipules small, early caducous; leaf blade pinnate-veined, sometimes 3–5-veined from the base; margin glandular-toothed, rarely entire. Inflorescences axillary, or terminal, on abbreviated lateral twigs, usually short, lax, racemose, or in the form of small paniculate or umbel-like clusters. Flowers hypogynous, unisexual or bisexual, small; pedicels articulate. Sepals 4–7, imbricate, slightly connate at base, green, small. Petals absent. Disk fleshy, entire, or comprised of different glands. Staminate flowers: stamens many, exserted, filaments free, filiform; anthers ellipsoid, small, versatile, longitudinally dehiscent, connective not

projected beyond thecae; disk extrastaminal; abortive ovary much reduced or absent. Pistillate flowers: disk surrounding the base of ovary; ovary superior, globose, ovoid, or bottle-shaped, incompletely 2–8-loculed by false septa; placentas 2-ovuled; styles isomerous with placentas, free or united, columnar; stigmas slightly dilated, flattened, reniform, recurved; staminodes usually absent. Fruit a berrylike indehiscent drupe with pyrenes two times as many as styles, globose, in dried material characteristically longitudinally angled, squarish or rectangular in longitudinal cross-section, with flattish apex and base, contracted or not at the equator, disk persistent at the bottom, style or stigma remnants persistent at the apex. Seeds ellipsoid, compressed.

1.7.2. *Flacourtia jangomas* (Lour.) Raeusch

Kingdom: Plantae

Clade: Tracheophytes

Clade: Angiosperms

Order: Malpighiales

Family: Salicaceae

Genus: *Flacourtia*

Species: *F. jangomas*

F. jangomas (Lour.) Raeusch, commonly known as Paniala or Indian plum, or Coffee plum, belongs to the family Flacourtiaceae (now placed in Salicaceae). It is a small deciduous tree growing up to 6-10m but occasionally reaching up to 14m in height. Trunks and branches of old trees are thornless, whereas woody thorns are present when young. The bark is light brown to copper-red or pinkish buff, flaking into thin, smooth, and lenticellate lamels. Young branches are white dotted by numerous suborbicular lenticels,

puberulous or mostly glabrous. Petiole is 6.0-8.0mm long, and leaves are alternate, deciduous, pale pink when young, spirally arranged, rarely ovate-lanceolate, long point toothed, very thin, both surface glossy, blade elliptic, serrate, 7.0cm-11.0cm x 3.5cm-4.0 cm, papery and show 3-6 pairs of secondary nerves. The inflorescence is axillary racemes 1.0-2.0cm long, sub corymbose, glabrous, few-flowered, the male 1.5-3.0cm and the female 1.0-1.5 cm long. Flowers are dioecious, white to greenish, comprising 4 or 5 ovate triangular petals bearing the fragrance of honey before or with the young foliage. Pedicels are very slender, 0.5-1 (-1.5 cm). Sepals 2 mm, 4-5 in number, are ovate, obtuse, greenish, and pubescent on both sides. The disk is fleshly entire or slightly lobed and is white or yellow (orange) colored. Male flowers are filaments, glabrous, and solitary or in clusters, while female flowers are solitary. Male and female flowers are on separate trees. The androecium consists of many anthers, which are ovate to suborbicular. Ovary is 4-6 celled, with two ovules per locule which are initially flask-shaped, soon sub globular, with 4-6 styles connate into a distinct, 1 mm high column, not or slightly free at their apices, each bearing a dilate, bilobed, recurved stigma. Flowers appear from December to April together with new leaves with a very beautiful fresh green colour. Fruits are ellipsoid berries, sub globose, 1.5-2.5cm in diameter, dull brownish red or purple, then blackish, with greenish-yellow pulp ripening from March to July, enclosing 4-5(-10) flat seeds. Seeds are tipped with the single, short style column with 4–6-minute stigma points. The tree is propagated through seeds. However, seeds are slow to germinate; therefore, propagation is usually by inarching or budding onto self-seedlings. Birds eat ripe fruits and are widely dispersed, thus facilitating a very wide distribution of the species (Sasi *et al*., 2018).

1.7.3. *Flacourtia indica* (Burm.f.) Merr.

Kingdom: Plantae

Clade: Tracheophytes

Clade: Angiosperms

Order: Malpighiales

Family: Salicaceae

Genus: *Flacourtia*

Species: *F. indica*

Flacourtia indica is a tree or shrub usually 3-5m tall, sometimes 10m. The bark is usually pale, grey, and powdery, and may become brown to dark grey and flaking, revealing pale orange patches. Leaves red or pink when a young, variable in size, oval to round, to 12 cm, edge toothed, becoming leathery; 4-7 pairs of veins clear on both surfaces; stalk to 2 cm. Flowers unisexual or occasionally bisexual (1 or several branches of a female specimen with perfect flowers, which, however, bear fewer stamens than the males). Male flowers in axillary racemes 0.5-2 cm long; pedicles slender, maybe pubescent, up to 1 cm long, the basal bracts minute and caducous. Sepals (min. 4) 5-6 (max.7), broadly ovate, apex acute to rounded, pubescent on both sides, 1.5-2.5 mm long and broad. Filaments 2-2.5 mm long; anthers 0.5 mm long. Disk lobulate. Female flowers in short racemes or solitary; pedicles up to 5 mm. Disk lobulate, clasping the base of the ovoid ovary; styles 4-8, central, connate at the base, spreading, up to 1.5 mm long; stigmas truncate. Fruit globular, reddish to reddish-black or purple when ripe, fleshy, up to 2.5 cm across, with

persistent styles, up to 10-seeded. Seeds 5-8, 8-10 x 4-7 mm; testa rugose, pale brown (Chatterjee *et al*., 2015).

1.7.4. *Flacourtia montana* J. Grah.

Kingdom: Plantae

Clade: Tracheophytes

Clade: Angiosperms

Order: Malpighiales

Family: Salicaceae

Genus: *Flacourtia*

Species: *F. montana*

Thorny trees to 8 m, located in evergreen and semi-evergreen forests. Thorns are about 5 cm long, dark grey, thin, and smooth. Leaves simple, alternate, spiral; petiole 5-10, stout, glabrous; lamina 12-22 x 6-8, ovate, elliptic, base acute or rounded, apex acute or acuminate, margin crenate-serrate, coriaceous, glabrous except midrib below, shining above; 3-5 nerves from the base, prominent, lateral veins 4-6 pairs, pinnate, prominent, intercostae scalariform, slender, prominent. Flowers unisexual, small, in axillary congested pubescent cymes; sepals 4 or 5 tomentose, small, imbricate; petals absent; stamens many; anthers versatile; ovary superior, urn-shaped, glabrous, incompletely 2-5 locular, ovules 2 in each cell; styles 5, reflexed, notched at the tip. Fruit a berry, globose, obtusely ribbed, 1-1.5 cm across, bright red, of a pleasant acid flavor; seeds few, reddish. It starts flowering and fruiting in the month of April-June. Endemic to the Western Ghats, common trees are found in South, Central, and south Maharashtra Sahyadris.

(https://sites.google.com/site/efloraofindia/species/m-z/s/salicaceae/flacourtia/flacourtia-montana).

Identification of material with male flowers is a challenging task. *F. indica* (as defined here) by its leaf size and shape. The remaining species are much more complex, at least from herbarium material, as staminate flowers seem to offer no valuable characters; leaves on flowering specimens are often young and, therefore, generally small, and in all these species, the leaf shape and size are variable, with character states overlapping between the species. *Flacourtia jangomas* usually have ovate to ovate-elliptic or, more rarely, ovate-lanceolate leaves. Most flora keys rely heavily on style characters to distinguish species. Staminate herbarium material might easily be misidentified. A molecular study based on fertile material could help resolve this problem (Flora of Tamil Nadu, VOL. I, 1983).

The diversity of the genus is between 15 and 17 species in tropical Africa and Asia and five species (one endemic) in China. *Flacourtia* spp., are often cultivated and harvested for fruit, medicinal use, or wood. Several species, especially *F. indica*, are cultivated as ornamentals for their fruits. The trunks of small trees are often guarded by branching spines (Everett and Thomas 1981). Male flowers of *Flacourtia* are easily confused with those of Xylosma; female flowers of the two genera are easily distinguished by style and stigma morphology, and young fruits by style morphology and internal structure (https://sites.google.com/site/efloraofindia/species/m---z/s/salicaceae/flacourtia)

1.8. Commercial Use for its Pharmacological Potentials

These plants are found to be enriched with various phytoconstituents, thus enabling their wide properties for industrial use (Ahmad, 1984). The various active constituents like glucoside ester, butyrolactone lignan disaccharide, fixed oils, coumarins, terpenoids

alkaloids, phenolic compounds, flavonoids tannins, sucrose, and proteins have been isolated from the plants. Two limonoids, namely limolin and jangomolide were reported in the stem and bark parts of *F. jangomas.* Bioactive compounds such as tremulacin, hydnocarpic acid, and corymbulosine are reported (Pandey and Dubey 2014). This plant has been reported as an effective remedy for the treatment of a variety of diseases. Fruits are used as appetizing and digestive, in jaundice and enlarged spleen (Kirtikar, 1998). Barks are used for the treatment of intermittent fever. Roots are used on nephritic colic and gum in cholera. The leaves range from 11mm to 9 mm and are useful in pruritis and scabies. Previous phytochemical investigation on this plant resulted in the isolation of β - sitosterol (a well-known phytosterol), β-sitosterol- β-D-glucopyranoside, ramontoside, butyrolactonelignan disaccharide, and flacourtin. *Flacourtia indica* (Flacourtiaceae) is India's most valuable traditional medicinal plant. It is now considered a valuable source of unique natural products for the development of medicines and targeting various diseases. Although different parts (leaves, bark, stem, fruits, root, and even whole plant) of the *Flacourtia indica* have been demonstrated for several pharmacological activities (Chitravadivu *et al*., 2009), the potential of root extract of this plant is yet to be resolved; therefore, the potential of the root extract was evaluated for pharmaceutical and antibacterial activity (Tyagi, 2010).

CHAPTER 2:
REVIEW OF LITERATURE

REVIEW OF LITERATURE

The relevant literature on the present investigation has been briefly reviewed from various studies carried out by the researchers to understand the different parameters of our research for the mentioned objectives.

2.1. Phytochemical analysis

Kermasha *et al*. (1987) showed the presence of proteins, vitamin C, fructose, α, β glucose, sucrose, calcium, potassium, phosphorus, iron, and magnesium based on the dry weight of *Flacourtia jangomas.* Ghani (2003) reported that *Flacourtia jangomas* constitute different chemical groups, including tannins, carbohydrates, fats (palmitic, hexadecadienolic, stearic, oleic, and linoleic acids), minerals, ascorbic acids, tartaric acids, proteins, amino acids, and phenolic compounds.

Singh and Singh (2010) reported the presence of carbohydrates, protein, lipids, alkaloids, glycosides, and tannins through phytochemical screening of the *Flacourtia jangomas* plant. Tannins can be found primarily in barks, but leaves and young shoots are also rich in tannins. Singh *et al*. (2010) and Talukdar *et al*. (2012) revealed that the leaves and stem of *Flacourtia jangomas* contain secondary metabolites such as carbohydrates, steroids, tannins, saponins, and phenolic acid, and flavonoids like quercetin, luteolin, and rutin.

Yadav and Munin (2011) for their study, used seven medicinal plants such as *Bryophyllum pinnatum, Ipomea aquatica, Oldenlandia corymbosa, Ricinus communis, Terminalia bellerica, Tinospora cordifolia, and Xanthium strumarium,* to investigate the

presence of phytochemicals and to determine the total phenolic and flavonoid contents of the selected medicinal plants. Soxhlet apparatus was used for the organic solvent extraction. The solvents used were water, methanol, ethanol, and acetone. Total phenolic contents of the aqueous extracts of the plants were determined by the Folin Ciocalteus reagent method, whereas total flavonoid contents of the aqueous extracts were determined by the aluminum Chloride method. Proteins, carbohydrates, phenols, tannins, flavonoids, and saponins, were detected in all tested plants. Total phenolic contents obtained were 71.6mg/g in *Xanthium strumarium* (Leaves) of the extract, and total flavonoid contents obtained were a maximum of 42.8mg/g in *Tinospora cordifolia* (Leaves). Their findings provided evidence that these tested plants' crude aqueous and organic solvent extracts contain medicinally critical bioactive compounds and justified their use in traditional medicines to treat different diseases.

Anjali and Sheetal (2013) evaluated the phytochemical constitution of methanolic extract from dried leaves of four medicinally important herbs *Ocimum sanctum, Mentha spicata, Trigonella foenum-graecum, Spinacia oleracea* utilized in the routine diet along with one important medicinal tree *Gmelina arborea*. Qualitative analysis of phytochemical constituents such as tannins, phlobatannins, saponins, flavonoids, steroids, alkaloids, quinones, coumarin, terpenoids, and cardiac glycosides and quantitative analysis of total phenolics, alkaloids, saponins, and flavonoids was performed by the well-known tests protocol available in the literature. The qualitative phytochemical screening revealed the extract richness in tannins, phlobatannin, saponins, flavonoids, steroids, and alkaloids, while the quantitative analysis of phenolics, alkaloids, saponins, and flavonoids revealed that *Mentha spicata* possessed maximum phenolic (18.41%), *Gmelina arborea* highest

alkaloids (5.66%) & flavonoids (22.80 %) and *Trigonella foenum-graecum* highest saponin (50.12%) contents.

Eramma and Gayathri (2013) performed the antibacterial potential and phytochemical analysis of *Flacourtia indica* root extract. Phytochemical screening of the root extract was done to determine the phytochemicals in the methanol extract. The methanolic extract of *F. indica* was screened for the range of phytochemicals by thin-layer chromatography, and the bioactive compound was identified using GC-MS. Results showed that the root extract's phytochemical screening revealed the presence of flavonoids, saponins, alkaloids, tannins, terpenoids, glycosides, and phenolic compounds. Their findings indicated that the root extract of *F. indica* possesses pharmacological activity and the potential to develop natural products based on pharmaceutical drugs also, this study justifies the use of the plant in folk medicine.

Kalpana *et al*. (2014) deeply emphasized finding the presence of phytochemicals in the aqueous, ethanol, and petroleum ether extracts of four ferns *Actinopteris radiata, Drynaria quercifolia, Dryopteris cochleata,* and *Pityrogramma calomelanos* by both qualitative and quantitative screening methods. In their qualitative analysis, the phytochemical compounds such as Tannins, Saponins, Flavonoids, Quinones, Phenols, Terpenoids, Alkaloids, Glycosides, Cardio glycosides, Coumarins, Betacyanin, Anthocyanin, Steroids were screened. While in quantitative analysis, the phytochemical compounds such as Total Tannin and Total Phenol were quantified. They found that the ethanolic fern extract performed well to show positivity rather than aqueous and petroleum ether fern extracts for the 13 phytochemical qualitative tests. Ethanolic extract showed strong positivity for all 13 phytochemical tests. They reported that the aqueous fern extract showed positivity for the phytochemical tests in the four ferns, while petroleum ether fern

extracts showed almost negativity in the four fern species studied. They also noted that the ethanolic fern extract showed strong positivity for phenol and tannin in all four species. Their quantitative analysis tested important secondary metabolites for Anthelmintic activity, such as total phenol and tannin content. They concluded that the ethanolic fern extract of total tannin and phenol content was highest in *Pityrogramma calomelanos* and least in *Drynaria quercifolia*.

Sahira and Cathrine (2015) dealt with the general techniques involved in the phytochemical analysis of plants. They emphasized the proper method for collecting plants, the extraction of active compounds from the various parts of plants. Detailed standard methods used for qualitative and quantitative analysis of the phytochemicals were noted. Their study served as a guide for carrying out the phytochemical analysis of plants and their parts. Dutta and Borah (2017) carried out a phytochemicalAnalysis on methyl alcoholic extract of *Flacourtia jangomas* fruits and reported the presence of secondary metabolites flavonoids, phenols, tannins, terpenoids, and saponins except for alkaloids.

Roghini and Vijayalakshmi (2018) evaluated the phytochemicals using quantitative and qualitative analysis of ethyl acetate, ethanol, n-hexane, and aqueous extracts with the help of standard techniques. Their quantification and phytochemical screening findings showed alkaloids, flavonoids, reducing sugars, Phenols, proteins, amino acids, saponins, tannins, terpenoids, and glycosides. Further, their study findings revealed that the ethanolic extract of fruit extract had more constituents than other extracts by quantitative method. They reported that the ethanolic extract of *Citrus paradisi* showed many compounds and thus concluded that it may have been used in traditional medicine to prevent several diseases.

Senthil *et al*. (2018) aimed to evaluate the potential of ethanol and aqueous extract of *F. jangomas*. They used shade-dried, pulverized leaves of *F. jangomas* to extract with ethanol and water separately and subjected them to qualitative and quantitative phytochemical analysis using standard procedures. Qualitative phytochemical analysis indicated the presence of glycosides, alkaloids, phytosterols, tannins, flavonoids, and phenolic compounds. Marimuthu and Subramaniyan (2022) studied phytochemical components using *Flacourtia indica* leaves in three different extracts (petroleum ether, chloroform, and ethanol). After the phytochemical screening, they found steroids, triterpenes, alkaloids, phenols, flavonoids, saponins, and tannins in the crude extract.

2.1 Biosynthesis of Nanoparticles

Jiale Huang *et al*. (2007) Biosynthesized nanoparticles of silver and gold from the novel sun-dried *Cinnamomum camphora.* They reported that not only could silver nanoparticles ranging from 55 to 80nm in size be fabricated, but triangular or spherical-shaped gold nanoparticles could also be easily modulated by reacting the novel sundried biomass of *Cinnamomum camphora* leaf with aqueous silver or gold precursors at ambient temperature. The marked difference in shape control between gold and silver nanoparticles was attributed to the comparative advantage of protective and reductive biomolecules. They reported that the polyol components and the water-soluble heterocyclic components were mainly responsible for reducing silver ions or chloroaurate ions and stabilizing the nanoparticles, respectively. The sundried leaf in this work was very suitable for the simple synthesis of nanoparticles.

Narayanan and Sakthivel (2008) carried out the extracellular biological synthesis of gold nanoparticles by a simple biological procedure using coriander extract as the reducing agent. They reported that aqueous gold ions were reduced when exposed to coriander leaf extract, resulting in the biosynthesis of gold nanoparticles in size range from 6.75–57.91nm. The gold nanoparticles were characterized by UV-Vis spectroscopy, X-ray diffraction (XRD), energy dispersive X-ray analysis (EDAX), Fourier transform infrared spectroscopy (FT-IR), and transmission electron microscopy (TEM).

Singh *et al*. (2012) worked on the extracellular biosynthesis of gold nanoparticles using *Padina gymnospora* to form gold nanoparticles in a short duration rapidly. The U.V.–vis spectrum of the aqueous medium containing gold ion showed a peak at 527 nm corresponding to the plasmon absorbance of gold nanoparticles. Scanning electron microscopy showed the formation of well-dispersed gold nanoparticles. FTIR spectra confirmed that hydroxyl groups present in the algal polysaccharides were involved in the gold bioreduction. AFM analysis showed the results of particle sizes (53–67 nm) and the average height of the particle roughness (60.0 nm). X-ray diffraction (XRD) spectrum of the gold nanoparticles exhibited Bragg reflections corresponding to gold nanoparticles. They concluded that the synthesized nanoparticles could be used for various pharmacological applications.

Pandey *et al*. (2013) performed a low-cost, eco-friendly method for the synthesis of gold nanoparticles (AuNPs) using guar gum (G.G.) as a reducing agent. The nanoparticles obtained by their study were characterized using UV–vis spectroscopy, scanning electron microscopy (SEM), transmission electron microscopy (TEM), and X-ray diffraction (XRD). Based on their results, a potential mechanism for this method of

AuNP synthesis was discussed. G.G./AuNPs nanocomposite (G.G./AuNPs NC) was exploited as an optical sensor for detecting aqueous ammonia based on surface plasmon resonance (SPR). It was found to have good reproducibility, response times of ~10 s, and excellent sensitivity with a detection limit of 1 ppb (parts-per-billion). Thus, this system allowed them to rapidly produce an ultra-low-cost G.G./AuNPs NC-based aqueous ammonia sensor.

Renata and Jolanta (2015) reported the synthesis of ZnO nanoparticles using *Trifolium pratense* flower extract. They characterized the synthesized ZnO nanoparticles through various techniques such as UV–Vis absorption spectroscopy, X-ray diffraction (XRD), Fourier transform infrared spectroscopy (FT-IR), and scanning electron microscopy (SEM) with Energy dispersive X-ray analysis (EDX).

Shobha *et al*. (2017) synthesized copper nanoparticles and stabilized them using leaf extract from the medicinal plant *Flacourtia montana*. The preliminary phytochemical screening of leaf extract showed the presence of sugar, proteins, amino acids, and secondary metabolites like tannins, saponins, and flavonoids. The UV Vis spectrum, SEM images, XRD, FTIR, and TEM analysis provided evidence for the biosynthesis of Cu_2O nanoparticles. They observed that Cu_2O showed remarkable antibacterial activity against *Xanthomonas vescatoria* among the bacterial strains under study and antifungal activity against *Alternaria solani*. They concluded that the Cu_2O showed dose-dependent antimicrobial activity against plant pathovars under investigation.

Nandhini *et al*. (2018) employed an anticancer plant, which is used in the Indian system of medicine *Flacourtia indica* to develop the AgNPs and revealed their anticancer potential in the Dalton Lymphoma Ascites (DLA) cell line model. Among the solvent extracts of *F*. *indica*, methanol extract was found to contain a higher level of yield; hence the preparation of methanolic extract from *F*. *indica* was optimized using Response Surface Methodology (RSM), which indicated that 88% of methanol concentration, 50°C of temperature and 88 min of extraction time results in higher phenolic yield. The optimized *F*. *indica* extract strongly reduced the silver into AgNPs. The synthesized AgNPs were characterized using Ultraviolet-visible spectroscopy scanning (major peak at 455nm), transmission electron microscopy (particle size of 14–24 nm), and zeta potential (−15mV).

Farooq *et al*. (2020) employed the development of silver nanoparticles (Fj-AgNPs) using an aqueous extract of the ripe fruit of *Flacourtia jangomas* as a reducing agent. The developed Fj-AgNPs were compared with ammine-modified silver nanoparticles (Am-AgNPs), developed through a chemical route using the reported approach, and confirmed the phytoreduction of Ag^{+} to Ag^{0}. Their work reported U.V.–visible spectrum peak at 418 nm and FTIR peak profile (at 1587.6, 1386.4, and 1076 cm^{-1} with corresponding compounds) in addition to the diffraction peak at 38°, 44°, 64°, and 78° in PXRD spectrum confirmed the synthesis of Fj-AgNPs with 8.29 nm average crystallite size.

Nichodimus *et al*. (2022) for the first time, synthesized $P{-}ZrO_2CeO_2ZnO$ nanoparticles using phytochemical extracts from *Flacourtia indica* leaves and applied in the photocatalytic degradation of Congo Red in the presence of Light Emitting Diode warm white light. The photocatalytic degradation was optimized with respect to

$P{-}ZrO_2CeO_2ZnO$ nanoparticle dosage, initial Congo Red concentration, and degradation time. Their work reported that leaves extract dosage, pH, and metal concentration had the most significant effects on the synthesis of the nanoparticles. Their investigation outcomes demonstrated that $P{-}ZrO_2CeO_2ZnO$ nanoparticles offered a high potential for photocatalytic degradation of Congo Red.

2.2 Antimicrobial activity

2.2.1 Antimicrobial activity of plant extracts

Akpan and Morgan (2002) evaluated methanolic extracts of different parts of *F. jangomas* plant, such as leaf, flower, bark, and root, for their antifungal efficacy against *Candida tropicalis*. This resistant strain ranks second or third causative agent of many candidal infections and several oral diseases such as dental caries, endodontic infections, periodontal diseases, and oral candidiasis using Flucanazole as a reference standard. Pravin *et al*. (2011) studied the antibacterial activity of the crude extract of *F. jangomas* against both gram-positive and gram-negative bacteria, which showed good antibacterial activity against *Shigella shiga* and *Bacillus megaterium* and moderate activity against *Bacillus cerus* and poor action against *Escherichia coli.*

Sarkar *et al*. (2011) reported that the root extract's chloroform fraction had strong antimicrobial activity against pathogenic bacteria. Srivastava *et al.* (2012) observed fruit extract of the plant exhibiting good antimicrobial activity against *Pseudomonas aeruginosa, Klebsiella pneumonia,* and *E. coli.* Eramma and Gayathri (2015) presented their study on the antioxidant and antifungal activities of the methanol (MeOH) root extract of *F. indica*. They also performed antifungal activity against human fungal pathogens, *Aspergillus niger* and *Trichophyton mentagrophytes*. Compared to *Aspergillus*

niger, the root extract showed strong antifungal activity against *Trichophyton mentagrophytes*, generating a broader zone of inhibition reported. Clotrimazole, a common antifungal medicine, was used as a positive control in this investigation. Their work results concluded that F. indica appears to be a promising herbal option for treating a variety of fungal and bacterial infections, with high antioxidant capacity.

Shukla *et al*. (2015) reported bacterial endophytes FjF2 and FjR1 isolated from roots showed broad-spectrum antimicrobial activity against clinical pathogens gram-positive (*Staphylococcus aureus,*) and gram-negative (*E. coli, Pseudomonas* spp., *Proteus vulgaris, Klebsiella* spp.) bacteria and thus indicated promising antimicrobial activity of the bacterial endophyte isolated against human pathogenic bacteria. George *et al*. (2016) observed that the most significant or highest antifungal activity was shown by *F. jangomas* flowers as observed from the highest inhibition zones when compared to positive control.

2.2.2 Antimicrobial activity of nanoparticles

Akhilesh *et al*. (2010) reported a one-pot synthesis of spherical gold nanoparticles (52–22nm) and their capping with cefaclor, a second-generation antibiotic, without using other chemicals. The differently sized gold nanoparticles were fabricated by controlling the rate of reduction of gold ions in an aqueous solution by varying the reaction temperature (20–70ºC). Antimicrobial testing carried out showed that cefaclor-reduced gold nanoparticles have potent antimicrobial activity against both Gram-positive (*Staphylococcus aureus*) and Gram-negative (*Escherichia coli*) bacteria as compared to cefaclor or gold nanoparticles alone. The cefaclor-reduced gold nanoparticles were further coated onto poly(ethyleneimine) (PEI) modified glass surfaces to obtain antimicrobial

coatings suitable for biomedical applications and were tested against *E. coli* as an exemplar of activity. Results from a combined spectroscopic (FTIR) and microscopic study (AFM) suggested that the action of these novel particles is through the combined effort of cefaclor inhibiting the synthesis of the peptidoglycan layer and gold nanoparticles generating 'holes' in bacterial cell walls, thereby increasing the permeability of the cell wall, resulting in the leakage of cell contents and eventually cell death.

Narendhran and Rajeshwari (2015) focused on synthesizing Zinc oxide (ZnO) nanoparticles using an aqueous extract of *Lantana aculeata* leaf and assessed their effects on antifungal activity against the plant fungal pathogens. Synthesized nanoparticles were confirmed byultraviolet–visible spectroscopy, Fourier transforms infrared spectrometer, energy-dispersive X-ray spectrometer, X-ray diffractometer, Field-emission scanning electron microscopy, and high-resolution transmission electron microscopy. The antifungal activity of ZnO nanoparticles was determined using the well diffusion method. All the characterization analyses revealed that nanoparticles were highly stable and crystalline. *L. aculeata*-mediated ZnO nanoparticles were spherical with an average particle size of 12 ± 3 nm. Antifungal studies concluded that the maximum zone of inhibition was observed in *Aspergillus flavus* (21 ± 1.0 mm) and *Fusarium oxysporum* (19 ± 1.0 mm) at 100 μg ml^{-1} concentration. Their results indicated the benefits of using ZnO nanoparticles synthesized, for it could be effectively used as an antifungal agent in the environmental aspect of agricultural development.

Kashyap *et al*. (2017) studied the synthesis of AgNPs utilizing *F. jangomas* leaf extract and its potential antibacterial effect on pathogenic bacteria *E. coli*. The synthesized

AgNPs were found to be highly effective against the bacteria. Das *et al*. (2017) carried out a study on the antimicrobial effect of n- butanol extract of *F. jangomas* fruits and reported significant activity against *S. aureus* and *E. coli* compared to the standard drug, Chloramphenicol.

Solmaz *et al.* (2020) studied for the first-time green synthesis of AgNPs using stems and flowers of *Teucrium polium* L. After the collection and preparation of the aqueous extract, the production of AgNPs was performed. Finally, the antifungal activity of these nanoparticles was also studied on *Fusarium oxysporum* by colony formation assay. The production of AgNPs was identified by an absorption peak at approximately 450 nm using U.V.–Visible spectroscopy. The SEM images also revealed that the prepared AgNPs are particles with no agglomeration. Furthermore, the TEM images exhibited that the AgNPs were well dispersed, and had no evident aggregation. The antifungal analysis revealed that these AgNPs have antifungal activity.

Oves *et al*. (2021) employed their work on plant extract of *Conocarpus Lancifolius* fruits to synthesize silver nanoparticles, and it was further employed as an antimicrobial and anticancer agent. Nanoparticles were characterized by U.V.–visible spectrometer notable peak and FTIR analysis. The stability and crystalline nature of materials were approved by XRD analysis. The antifungal study was carried out against *Streptococcus pneumonia* and *Staphylococcus aureus*. The inhibition zones of 18 and 24 mm have been estimated to be antibacterial activity against both bacteria. Based on their results, the biogenic AgNPs revealed significant action against the fungal pathogen *Rhizopusus stolonifera* and *Aspergillus flavus*.

Sathiyaraj *et al*. (2021) used panchagavya (P.G.) to synthesize gold nanoparticles, and several spectroscopic techniques characterized the resulting nanoparticles (PG-AuNPs). In addition, they carried out the antibacterial activity of PG-AuNPs against *Escherichia coli*, *Bacillus subtilis*, and *Klebsiella pneumoniae* by a well-diffusion method. They observed that the antibacterial activity of PG-AuNPs was strong against gram-negative bacteria and moderate against gram-positive bacteria. Based on the result, they concluded that PG-AuNPs could be used to combat antibiotic drug resistance.

CHAPTER 3: OBJECTIVES

OBJECTIVES

Flacourtia is a genus comprising flowering plants in the family Salicaceae. It was previously placed in the now defunct family Flacourtiaceae ("Flacourtia Comm. ex L'Hér". 2006). The generic name honors Étienne de Flacourt (1607–1660), a governor of Madagascar. It contains 15 species of shrubs and small trees that are native to the African and Asian tropics and subtropics. Several species, especially *F. indica*, are cultivated as ornamentals and extensively relished for their fruits. The trunks of small trees are often guarded by branching spines. Since the genus is home to wild species it seeks the attention of researchers to exploit its innumerable properties.

The prime aim of this investigation was to provide insights into all the 3 species diversity found in Goa. *Flacourtia jangomas* a common garden tree found in the houses of Goans; *Flacourtia indica* which is present in fewer regions of Goa such as Canacona, and Socorro Plateau and *Flacourtia montana* found in the reserved areas of Canacona and remote parts of Sattari. These species are intended to study for the following experimental objectives: -

1) Qualitative and quantitative phytochemical analysis of leaves extract of the selected *Flacourtia spp.*
2) Green synthesis of gold nanoparticles and its physical characterization using UV-Vis spectrum, FTIR, SEM/EDS and XRD methods.
3) Antimicrobial activity of the green synthesized gold nanoparticles.

CHAPTER 4: MATERIALS AND METHODS

METHODS AND MATERIALS

3.1.1 Collection of Plant Materials

The mature and healthy leaves of the selected *Flacourtia* spp., *(Flacourtia jangomas, Flacourtia indica and Flacourtia montana)* were collected from different regions of Tiswadi and Canacona talukas of Goa (**Plate 1, 2, 3**). The collected leaves of the three different *Flacourtia* spp., were cleaned with tap water followed by distilled water and allowed to dry completely for 14 days under shade at room temperature (27±1ºC).

Table 1. Names of the collected species and their location

Sr. No.	Species Name	Location	GPS coordinates
1.	*Flacourtia jangomas* (Lour.) Raeusch.	Tiswadi, Goa	15.496255, 73.870013
2.	*Flacourtia indica* (Burm.f.) Merr.	Canacona, Goa	14.9804355 74.0820729
3.	*Flacourtia montana* J. Grah.	Canacona, Goa	14.9752922 74.1018518

3.1.2 Chemical Requirements

Chloroauric acid ($HAuCl_4$) (Analytical grade) was purchased from Sigma-Aldrich, India (99.9%) purity. Methanol was used as the extracting solvent. For phytochemical analysis the chemicals used were dilute hydrochloric acid, concentrated hydrochloric acid, chloroform, pyridine, sodium nitroprusside solution, chloroform, 10% sodium hydroxide, 2% copper sulfate solution, potassium hydroxide pellets, ninhydrin solution, acetic anhydride, concentrated sulphuric acid, phenolphthalein, 5% ferric chloride solution, 10% lead acetate solution, ethanol, Folin– Ciocalteau reagent, 7% Na_2CO_3, Gallic acid, aluminum chloride, Quercetin. The chemicals and media used for antimicrobial assay are

Malt extract agar (MEA) (HiVeg Media) media, Muller-Hinton Agar (MHA) media and streptomycin.

3.2 Preparations of Plant Extracts

The fresh leaves of *F. jangomas, F. indica, F. montana* were washed thoroughly under tap water to remove the dust particles and rinsed briefly in de-ionized water. Fresh and healthy leaves were put for drying under the shade. After 14 days of thorough drying, the leaves were powdered using a grinder and stored in air-tight containers for further use. The leaf extracts of each plant were prepared using the Soxhlet extraction technique for 24 hrs. 10g of each dry powdered material was subjected to extraction at 35°C with 150mL of the two solvents of different polarities i.e., methanol and water. Water extracts were filtered using a normal filter and Whatman Grade-A filter papers. Then it was placed at 60°C in the oven until completely dried to obtain the extract. Methanol extracts were concentrated using Rotary Vacuum Evaporator under reduced pressure at 40°C until to obtain solid residues. Obtained leaf extracts were stored in glass vials at -4°C for further analysis.

3.3 Preliminary Qualitative phytochemical analysis

The extracts obtained with two different solvents were used for the preliminary qualitative estimation for detecting the various phytoconstituents present in them and were carried out according to the methods described by Raaman (2006).

3.3.1 Detection of Alkaloids (Evans,1997)

50mg solvent-free extract was stirred with a few mL of dilute hydrochloric acid and filtered. The filtrate was tested with various alkaloidal reagents.

a. Wagner's Test

From the side of the test tube, a few drops of freshly prepared Wagner's reagent was added to a few mL of filtrate. The appearance of a reddish-brown precipitate indicates the presence of alkaloids.

b. Hager's Test

1-2mL of Hager's reagent was added to a few mL of filtrate. A prominent yellow precipitate reveals the presence of alkaloids.

3.3.2 Detection of Carbohydrates and Glycosides (Ramakrishnan *et al.*, 1994)

100mg free extract was stirred with 5mL of distilled water and filtered. The filtrate was subjected to various tests.

a. Barfoed's Test

To 1mL of filtrate, 1mL of barfoed's reagent is added and placed in boiling water bath for 2min. Red precipitate indicates the presence of carbohydrates.

b. Benedicts Test

0.5mL of the filtrate was added to 0.5mL of Benedict's reagent and placed in a boiling water bath for 2 minutes. Reddish brown precipitate indicates the presence of carbohydrates.

For detection of glycosides: 50mg of the extract is hydrolyzed with a few mL of concentrated hydrochloric acid for 2 hours in the water and filtered.

c. Borntrager's Test

3mL of chloroform is added to 2mL of filtrate and shaken. Then 10% ammonia solution is added to separate the chloroform layer. The appearance of pink color indicates the presence of glycosides.

d. Legal's Test

50mg solvent-free extract is dissolved in pyridine. Then few mL of sodium nitroprusside solution is added and made alkaline using 10% sodium hydroxide. The appearance of pink color indicates the presence of glycosides.

3.3.3 Detection of Saponins (Kokate, 1999)

50mg extract is dissolved in 10 mL of distilled water and the final volume is made up to 20mL and shaken for a few minutes**.** The appearance of a 2cm foam layer indicates the presence of saponins.

3.3.4 Detection of Proteins and Amino Acids (Fisher, 1968; Ruthmann, 1970)

100mg free extract was stirred with 10mL of distilled water and filtered. The filtrate was subjected to various tests.

a. Biuret Test (Gahan,1984)

A 2% copper sulfate solution drop is added to 2mL of filtrate. Then 1mL of 95% ethanol is added, followed by an excess of potassium hydroxide pellets. The appearance of pink color in the ethanolic layer indicates the presence of proteins.

b. Ninhydrin Test (Yasuma and Ichikawa, 1953)

To a few mL of filtrate 2 drops of freshly prepared ninhydrin solution are added. The appearance of purple color indicates the presence of amino acids.

3.3.5 Detection of Phytosterols (Finar, 1986)

50mg of the solvent-free extract is dissolved in 2 mL acetic anhydride. Then 1-2 drops of concentrated sulphuric acid are added slowly along the sides of the test tube. An array of color changes indicates the presence of phytosterols.

3.3.6 Detection of Fixed Oils and Fats (Kokate, 1999)

To a small quantity of extract, a few drops of 0.5N alcoholic potassium hydroxide are added along with a drop of phenolphthalein and heated in the water bath for 2hr. Soap formation indicates the presence of fixed oils and fats.

3.3.7 Detection of Phenolic Compounds and Tannins

a. Ferric chloride Test

In 5mL of distilled water, 50mg of the extract is dissolved. To this few mL of freshly prepared neutral 5% ferric chloride solution is added. The appearance of dark green color indicates the presence of phenolic compounds.

b. Lead acetate Test

50mg extract is dissolved in distilled water, and to this 3mL of 10% lead acetate solution is added. The appearance of a bulky white precipitate indicates the presence of phenolic compounds.

3.3.8 Detection of Gum and Mucilages (Whistler and BeMiller, 1993)

100mg of the extract is dissolved in 10mL of distilled water, and this 25mL of alcohol is added with constant stirring. The appearance of a white or cloudy precipitate indicates the presence of gums and mucilages.

3.4. Quantitative phytochemical analysis

3.3.9 Determination of total phenol

The Folin–Ciocalteau reagent method has been used for the estimation of total phenolic extracts quantities. 200µL sample extract of the plant had been taken and mixed with 300µL of Folin– Ciocalteau reagent. This mixture was allowed to stand for 5min at room temperature. Later 1mL of 7% Na_2CO_3 was added and the blend was incubated in the dark at room temperature for 1 hr. All samples' absorbance of the blue-colored solution

was measured at 765 nm. The results were expressed in mg of gallic acid equivalent (GAE) per g of the dry weight of plant powders.

3.3.10 Determination of total tannins

The sample's Tannin content was estimated by following the standard procedure (Hagerman *et al*., 2000). The sample extract (1mL) was mixed with Folin-Ciocalteau's reagent (0.5mL), followed by the addition of saturated Na_2CO_3 solution (1mL) and distilled water (8mL). The reaction mixture was allowed to stand for 30 min at room temperature. The absorbance of the blue-colored solution was recorded at 725 nm using UV-Visible Increasing concentrations of standard tannic acid were prepared and the absorbance of various (0, 20, 40, 60, 80, 100 µL) tannic acid concentrations was plotted for a standard graph. The tannin content was expressed as mg tannic acid equivalent per 100 grams of the sample.

3.3.11 Determination of total flavonoid

The aluminum chloride colorimetric method was used with some modifications to determine flavonoid content. 200µL of sample plant extract was mixed with 1.5mL of 95% ethanol, 0.1mL of 10% aluminum chloride, 0.1mL of 1M potassium acetate, and 2.8mL of distilled water and incubated at room temperature for 30 minutes. The absorbance was measured at 415nm. Quercetin was used as standard (1mg/mL). Flavonoid contents were determined from the standard curve and were expressed as quercetin equivalent (mg/g of extracted compound).

3.4 Preparation of Leaf extract for the biosynthesis of nanoparticles

10g of dry leaf powder of the selected three *Flacourtia* spp*.,* were dissolved in 300mL distilled water and boiled until bubbling started on the heating mantle at 100°C for 20 minutes in a screw cap bottle. The extract was filtered using normal filter paper and

Whatman Grade-A filter papers and stored at 4°C. The filtrate was used for the biosynthesis of the nanoparticles.

3.5 Biosynthesis of Gold Nanoparticles Using Leaf Extract

The leaf extract (5mL) was added to 400mL of 10−3 M $HAuCl_4$ aqueous solution and kept at 30°C. The color change after 30min, 1hr, 3hr, 5hr, and 24hr of the solution was monitored for 24hrs.

3.5.1 UV-Vis absorbance spectroscopy

The bio-reduction of $AuCl^{4-}$ ions in solution was monitored by periodic sampling of 2mL aliquots of aqueous component and measuring the solution on a UV-Visible spectrophotometer in the range of 400-800 nm.

For further characterization, the reduced nanoparticle solution was centrifuged at 10,000rpm at 15°C for 15 min. The supernatant was discarded, and the pellet was washed with 20mL distilled water and centrifuged again at 10,000 rpm. The prominent pellet formed was collected in a Petri plate and allowed to dry in the oven at 60°C to obtain the dry extract. The extract obtained was stored in Eppendorf tubes at room temperature for further characterization of Au nanoparticles.

3.5.2 Fourier transform infrared spectroscopy (FTIR)

1mg of the dried extract was ground with 100mg of KBr pellets and allowed to dry at 60°C in the hot air oven for 30 min. later it was analyzed on a Thermo Nicolet model 6700 spectrum one instrument in the diffuse reflectance mode operating at a resolution of 4 cm^{-1}.

3.5.3 Field Emission Scanning electron microscopy (FE-SEM)

The nanoparticles were mounted on the copper stubs, and the images were studied using a scanning electron microscope (SEM) EDS, Quanta FEG 250, with secondary

electron detectors at an operating voltage of 30 kV. Specific areas were located from SEM images and the elemental composition was recorded using EDS.

3.5.4 X-Ray diffraction (XRD) analysis

The bio-reduced chloroauric acid solution was drop-coated onto a glass substrate, and powder X-ray diffraction measurements was carried out on a Bruker D8 Advance powder diffractometer. The pattern was recorded by CuKα radiation with k of 1.5406A° and nickel monochromator filtering the wave at a tube voltage of 40 kV and tube current of 30 mA. The scanning was done in the 2h from 20 to 80° at 0.02° /min, and the time constant was 2s.

3.6 Antimicrobial assay

3.6.1 Fungal and bacterial species

The fungal strains (F*usarium* spp., *Aspergillus* spp., *Penicillium* spp.,) were obtained from the Goa University fungal culture collection lab (GUFCC), Department of Botany, Goa University. The bacterial (*Escherichia coli)* strain was used and maintained in saline solution at room temperature for experimental studies.

3.6.2 Antifungal assay

The well diffusion method carried out the dose-dependent antifungal activity of the synthesized Au NPs. The fungal strains were grown in Malt extract agar (MEA) media for 48h and used for the study. Microorganism was prepared by spreading 200µL of revived culture (containing 104 cells mL^{-1}) on Malt extract agar (MEA) (HiVeg Media) media with the help of a spreader. Various volumes of Au NPs (20, 60, and 100µL) were added to the centre of the well with 7mm of dia. The streptomycin and distilled water were used as a positive and negative control for the antifungal assay. The Petri plates were incubated at 25°C for 48h at room temperature.

3.6.3 Antibacterial assay

The well diffusion method carried out the dose-dependent antibacterial activity of the synthesized Au NPs. The bacteria were grown on Muller-Hinton Agar (MHA) media for 24h and used for the study. The microorganism was prepared by spreading 200µL of revived culture (containing 104 cells mL^{-1}) on Muller-Hinton Agar (MHA) media with the help of a spreader. Various volumes of Au NPs (20, 60, and 100µL) were added to the center of the well with 7mm of dia. The antibacterial assay used ampicillin and distilled water as positive and negative control. The Petri plates were incubated at 25°C for 24h at room temperature.

CHAPTER 5: RESULTS AND DISCUSSION

RESULTS AND DISCUSSION

4.1 Qualitative analysis of selected *Flacourtia* spp.

The result of the preliminary phytochemical screening carried out on the methanolic and water extracts of all three *Flacourtia* spp., samples revealed the presence of a wide range of phytoconstituents, including alkaloids, carbohydrates, glycosides, saponins, proteins, amino acids, phenolic compounds, and tannins, supporting the reason for its wide range of biological activities, as shown in **(Table 2, Plate 4, 5)**. Phenolic compounds showed a strongly positive result for *Flacourtia* spp., thus indicating a solid spread of these constituents in plant biology. Test for gums and mucilage was carried out, but the results were negative, indicating the absence of these constituents in all three *Flacourtia* spp.

4.2 Quantitative phytochemical analysis

The plants contain chemicals that are termed phytoconstituents, essential for their functioning. These phytoconstituents present universally can be classified as primary and secondary metabolites. Primary metabolites include proteins, amino acids, sugars, purines, and pyrimidines of nucleic acids, chlorophylls, etc., during secondary phytochemicals such as alkaloids, terpenoids, and different phenols.

A comparative study of Total Phenolic Content (TPC) on all methanolic and water leaf extracts was estimated using a modified Folin-Ciocalteu calorimetric method. Gallic acid was taken as standard, and absorbance was recorded at 765 nm **(Figure 1)**. A gradient color change of blue color was observed as the concentration of std varied. Among the samples analyzed, the methanolic extract of *F. montana* contained a maximum (911.51±0.005) compared to the other two methanolic leaf extracts. The minimum phenolic content was in *F. indica* (743.80 ± 0.056) methanolic extract. In water extract, *F. jangomas* showed

better results (908.39 ± 0.018) for phenolics than *F. indica and F. montana* **(Table 3)**. The total Tannin Content (TTC) on all methanolic and water leaf extracts was estimated using the modified Folin-Ciocalteu calorimetric method. Tannic acid was taken as standard, and absorbance was recorded at 725 nm **(Figure 1)**. A gradient color change of blue color was observed as the concentration of std varied. Total Tannin Content (TTC)for methanolic extract was found to be higher in *F. jangomas (*902.66 ± 0.006*),* followed by *F. montana* and *F. indica.* While water extracts, it was seen that *F. montana* showed higher phenolic content than the rest samples **(Table 4)**. The Aluminum-chloride calorimetric method estimated the total Phenolic Content (TPC) on all methanolic and water leaf extracts. Quercetin was taken as standard, and absorbance was recorded at 415 nm **(Figure 1)**. A gradient color change of yellow color was observed as the concentration of std varied. Total Flavonoid Content (TFC) for methanolic extract was again found to be maximum (645.21 ± 0.025) in *F. montana* and minimum (240.35 ± 0.004) in *F. indica*. For water extract, phenolics were the highest in *F. montana* **(Table 5)**. It may be concluded from the present study on the selected species that methanolic extract showed better results than the water extract **(Plate 6, Table 6, Figure 2)**.

Plate 1. ***Flacourtia jangomas*****; A) habit B) branched woody thorns C) twig D) leaf adaxial E) leaf abaxial.**

Plate 2. ***Flacourtia indica;*** **A) habit B) branched woody thorns C) twig D) leaf adaxial E) leaf abaxial.**

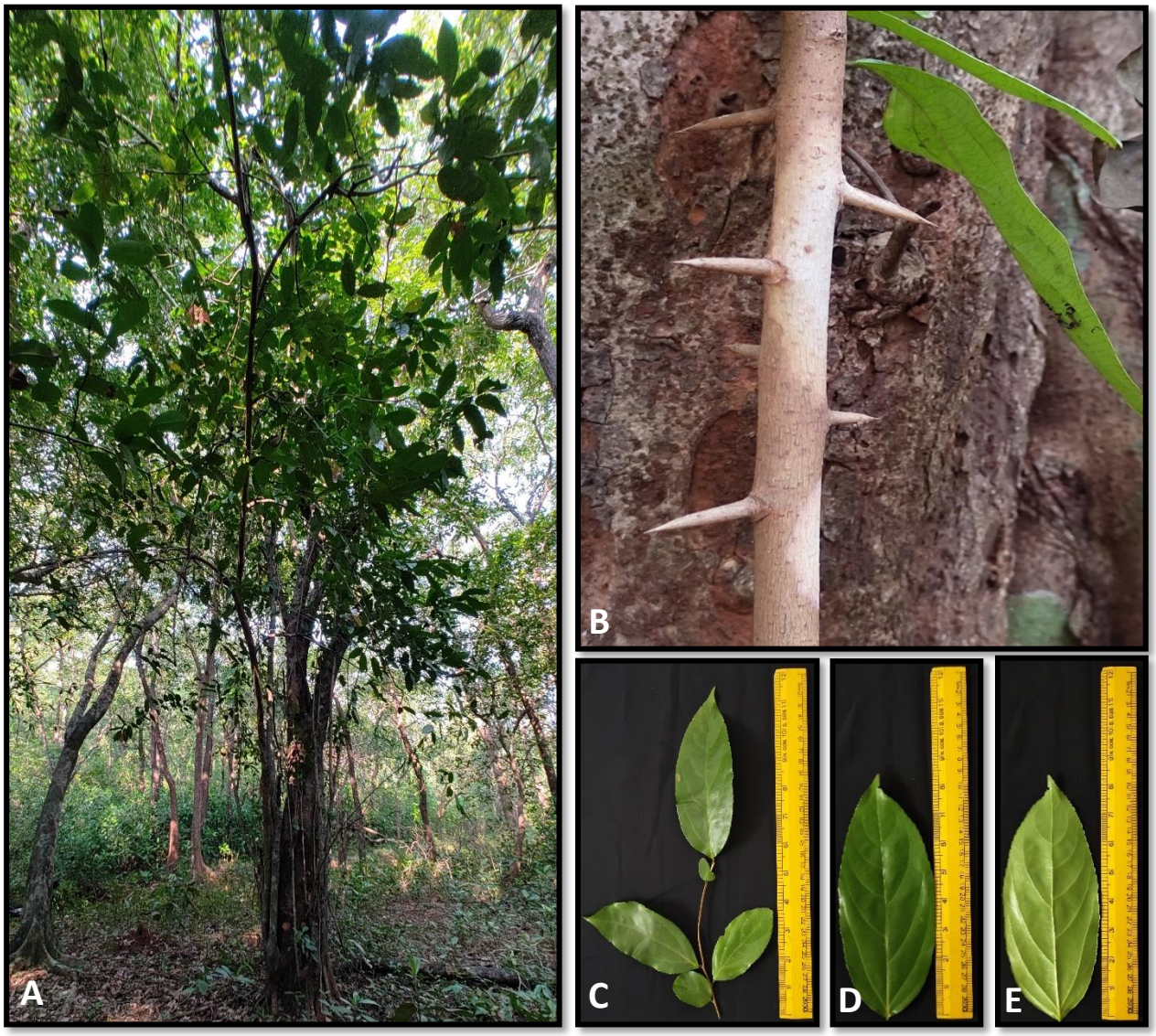


Plate 3. ***Flacourtia montana;*** **A) habit B) simple woody thorns C) twig D) leaf adaxial E) leaf abaxial.**

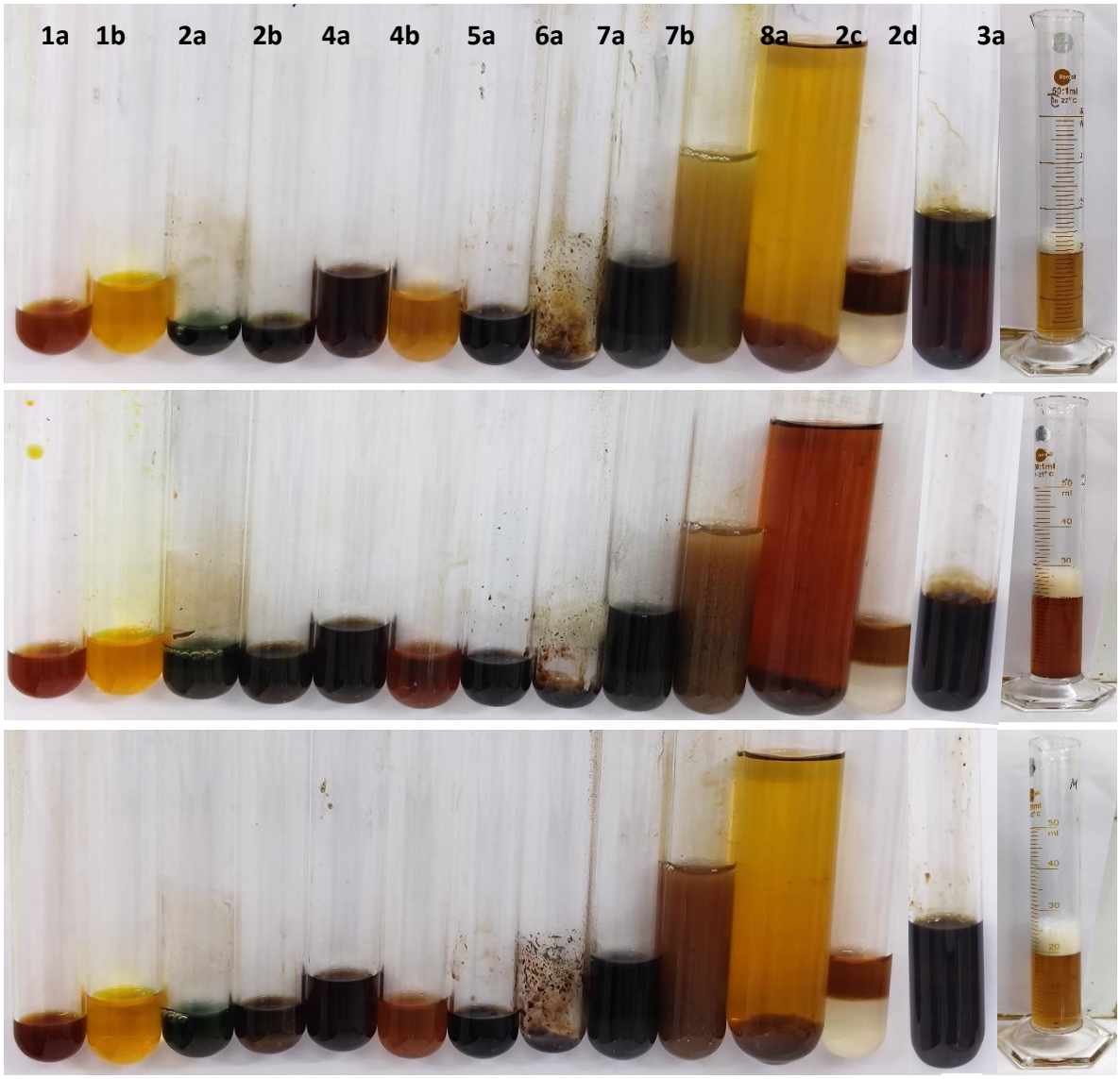


Plate 4. Qualitative Phytochemicals analysis of selected *Flacourtia* plant specimens using water as extracting solvent.

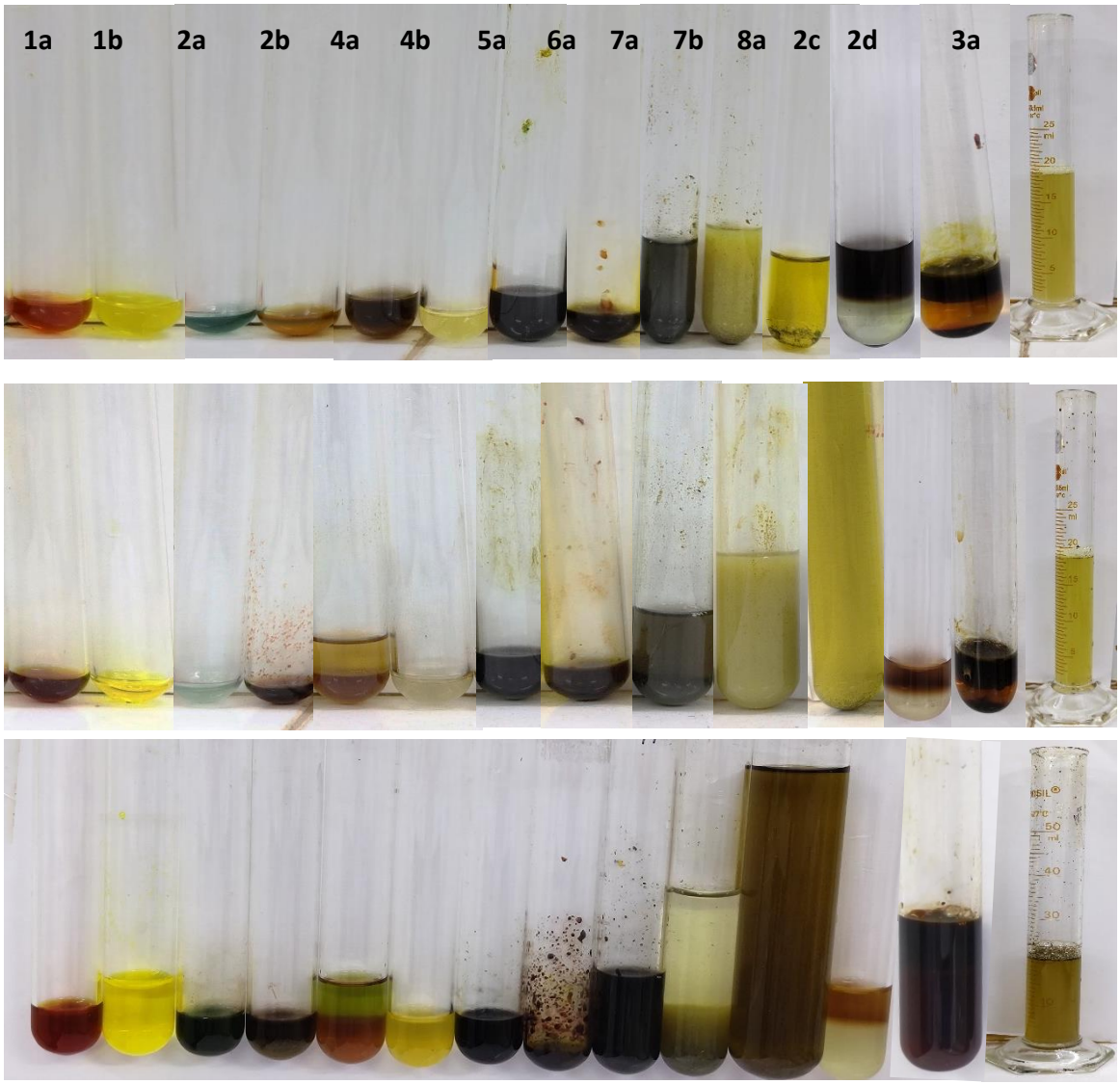


Plate 5. Qualitative Phytochemicals analysis of selected *Flacourtia* plant specimens using methanol as extracting solvent.

Table 2. Qualitative phytochemical analysis for *Flacourtia* spp., *F. jangomas, F. indica, F. montana* using water and methanol as the extracting solvents.

Sr. No.	Phytochemicals	Phytochemical test	*Flacourtia jangomas*		*Flacourtia indica*		*Flacourtia montana*	
			M/OH	D/W	M/OH	D/W	M/OH	D/W
1	**ALKALOIDS**	a) Wagner's test b) Hager's test	+ +	+ +	+ +	+ +	+ +	+ +
2	**CARBOHYDRATES & GLYCOSIDES**	a) Barfoed's test b) Benedict's test c) Borntrager's test d) Legal's test	+ + + +	+ + + +	+ + + +	+ + + +	+ + + +	+ + + +
3	**SAPONINS**	a) Saponin test	+	+	+	+	+	+
4	**PROTEINS & AMINO ACIDS**	a) Biuret test b) Ninhydrin test	+ -	+ -	+ -	+ -	+ -	+ -
5	**PHYTOSTEROLS**	a) Libermann-Burchard's test	+	+	+	+	+	+
6 7	**FIXED OILS & FATS**	Saponification test	-	-	-	-	-	-
8 9	**PHENOLIC COMPOUNDS & TANNINS**	a) Ferric-chloride test b) Lead acetate test	++ ++	++ ++	++ ++	++ ++	++ ++	++ ++
10 11	**GUMS & MUCILAGE**	1. Alcohol 95% test	-	-	-	-	-	-

+: Positive Result, ++ strongly positive result, -: Negative result

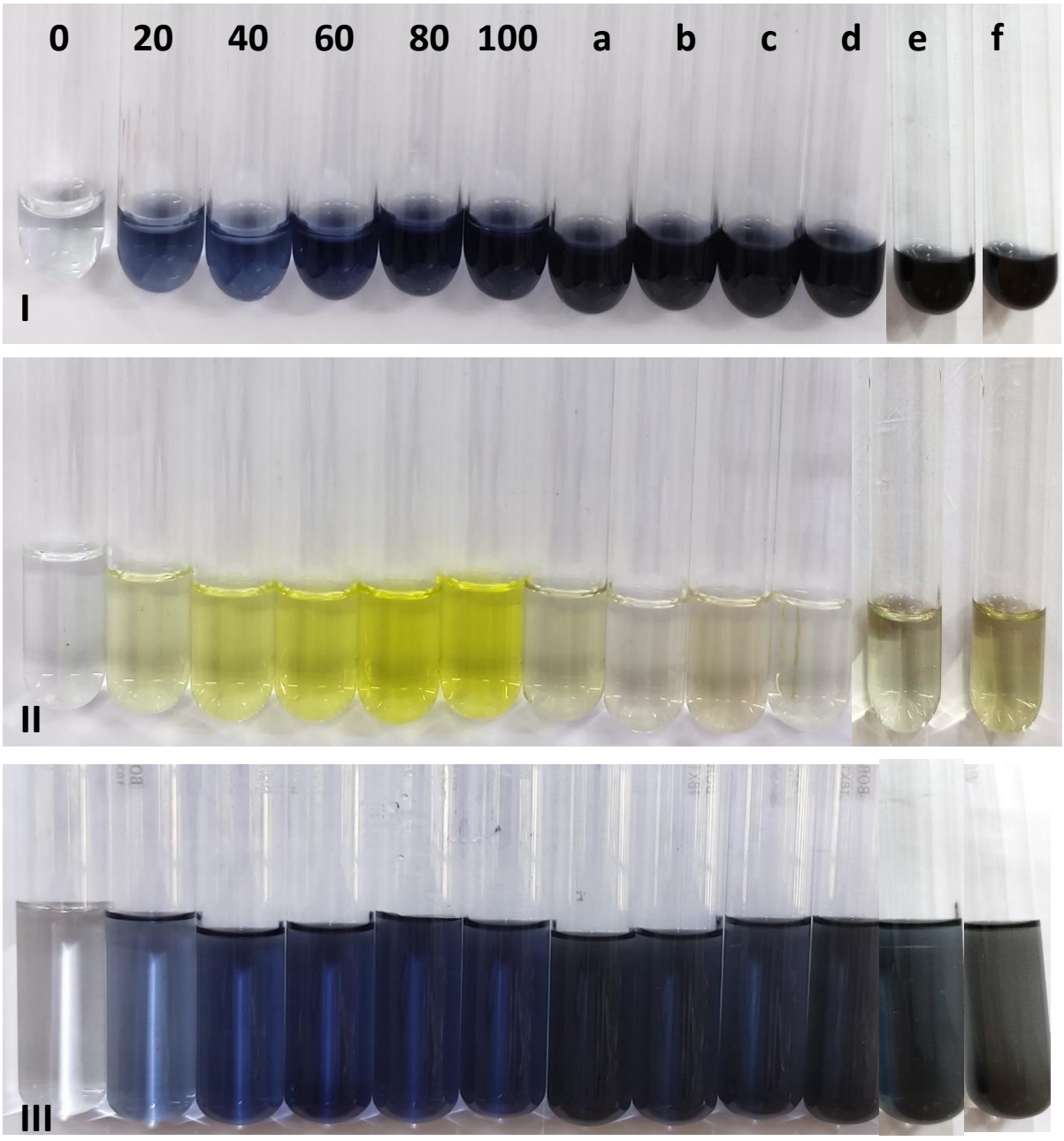


Plate 6. Quantitative Phytochemicals analysis of selected *Flacourtia* plant specimens using methanol (Me-OH) and water(D.W.) as extracting solvents.

I-Total Phenolic Content, II- Total Tannin Content, III-Total Flavonoid Content

a) *F. montana* Me-OH b) *F. montana* D.W *c) F. jangomas* Me-OH d) *F. jangomas* D.W *e) F. indica* Me-OH f) *F. indica* D.W.

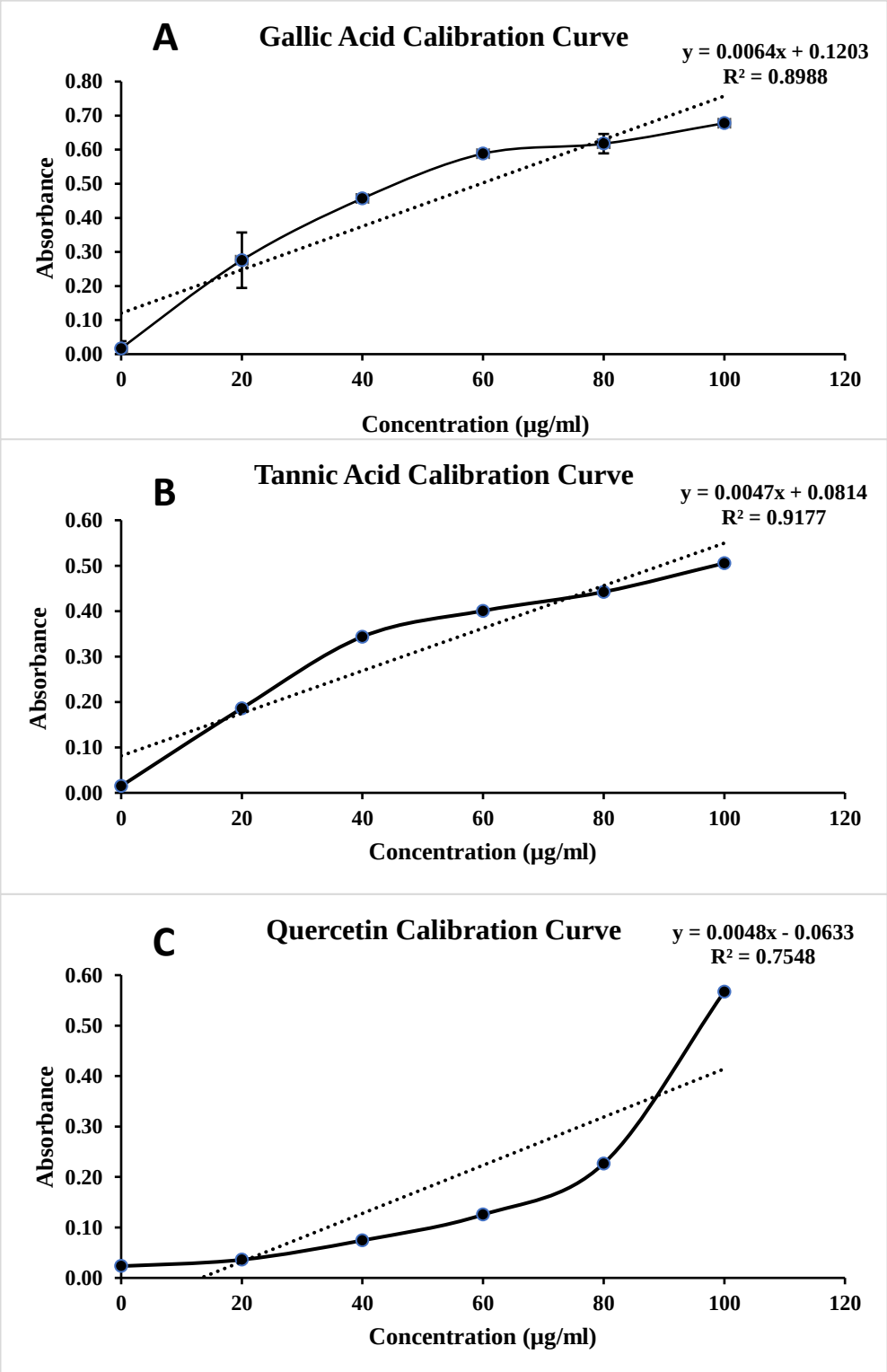


Figure 1. Calibration curves for (A) Gallic acid, (B) Tannic acid, (C) Quercetin

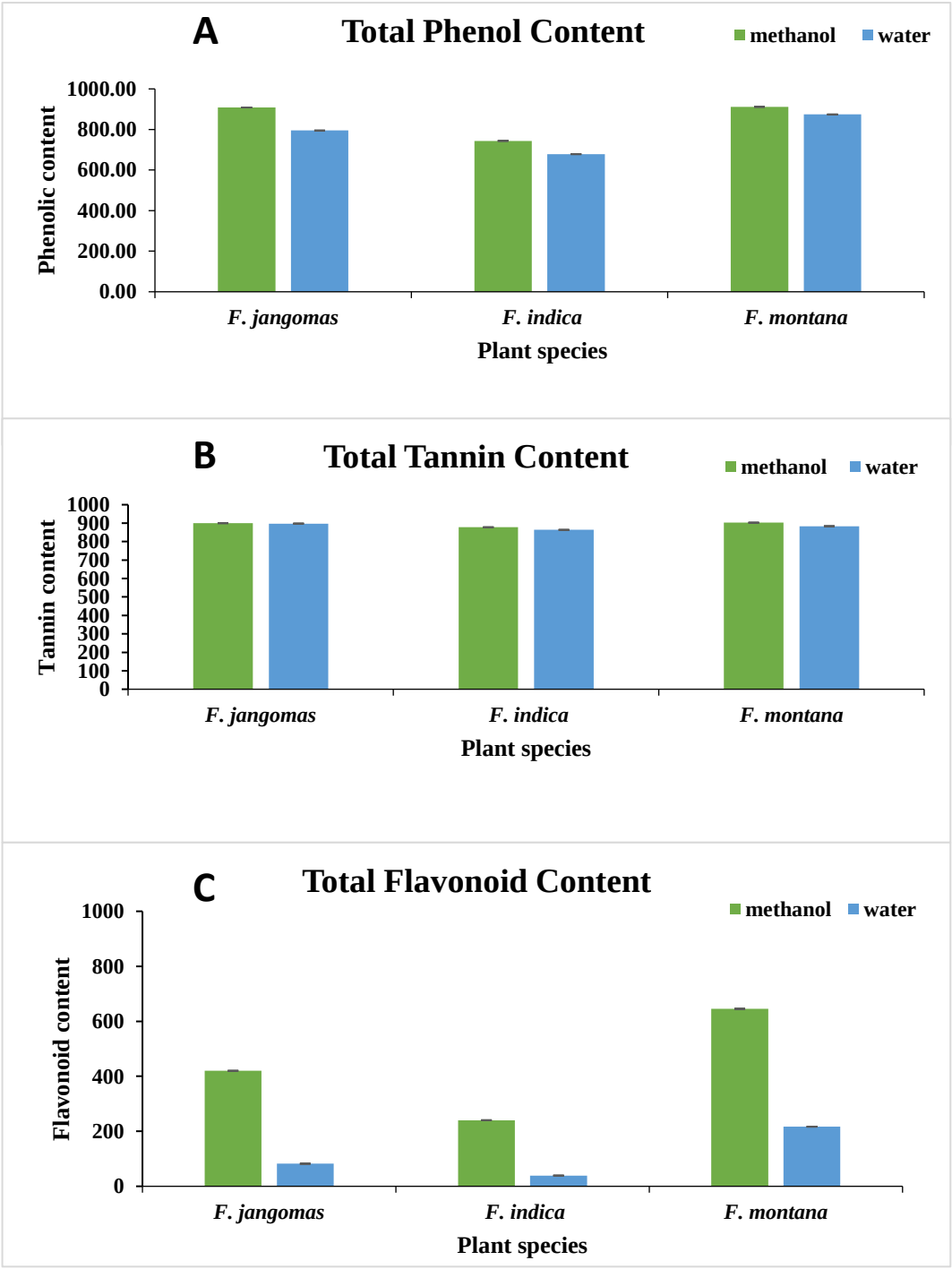


Figure 2. Comparative graph for (A) Total Phenolic Content, (B) Total Tannin Content, (C) Total Flavonoid Content

Table 3. Total Phenolic Content of the Selected *Flacourtia* spp.

Sr. No.	Concentration (µg/mL)	Absorbance at (765nm)			Mean	Standard Deviation
		i	ii	iii		
1	0	0.017	0.017	0.017	0.02	0.000
2	20	0.28	0.299	0.248	0.28	0.021
3	40	0.342	0.512	0.517	0.46	0.081
4	60	0.587	0.579	0.599	0.59	0.008
5	80	0.612	0.615	0.626	0.62	0.006
6	100	0.648	0.669	0.716	0.68	0.028
7	**Unknown samples**					
8	P.E. of *F. jangomas* (Me-OH)	0.573	0.68	0.634	0.63	0.044
9	P.E. of *F. indica* (Me-OH)	0.537	0.581	0.671	0.60	0.056
10	P.E. of *F. montana*(Me-OH)	0.698	0.703	0.71	0.70	0.005
11	P.E. of *F. jangomas* (H_2O)	0.64	0.668	0.683	0.66	0.018
12	P.E. of *F. indica* (H_2O)	0.529	0.556	0.578	0.55	0.020
13	P.E. of *F. montana* (H_2O)	0.678	0.636	0.713	0.68	0.031

Table 4. Total Tannin Content of the Selected *Flacourtia spp.*

Sr. No.	Concentration (µg/ml)	Absorbance at (725nm)			Mean	Standard Deviation
		i	ii	iii		
1	0	0.013	0.017	0.015	0.02	0.002
2	20	0.186	0.185	0.187	0.19	0.001
3	40	0.343	0.345	0.343	0.34	0.001
4	60	0.391	0.403	0.408	0.40	0.007
5	80	0.427	0.436	0.464	0.44	0.016
6	100	0.489	0.512	0.515	0.51	0.012
7	**Unknown samples**					
8	P.E. of *F. jangomas* (Me-OH)	0.498	0.513	0.506	0.51	0.006
9	P.E. of *F. indica* (Me-OH)	0.5	0.478	0.504	0.49	0.011
10	P.E. of *F. montana*(Me-OH)	0.507	0.514	0.489	0.50	0.011
11	P.E. of *F. jangomas* (H_2O)	0.503	0.493	0.494	0.50	0.004
12	P.E. of *F. indica* (H_2O)	0.472	0.489	0.501	0.49	0.012
13	P.E. of *F. montana* (H_2O)	0.506	0.499	0.508	0.50	0.004

Table 5. Total Flavonoid Content of the Selected *Flacourtia* spp.

Sr. No.	Concentration (µg/ml)	Absorbance at (415nm)			Mean	Standard Deviation
		i	ii	iii		
1	0	0.021	0.024	0.025	0.02	0.002
2	20	0.036	0.038	0.034	0.04	0.002
3	40	0.081	0.063	0.079	0.07	0.008
4	60	0.115	0.134	0.127	0.13	0.008
5	80	0.226	0.235	0.219	0.23	0.007
6	100	0.591	0.656	0.455	0.57	0.084
7	**Unknown samples**					
8	P.E. of *F. jangomas* (Me-OH)	0.286	0.239	0.273	0.27	0.020
9	P.E. of *F. indica* (Me-OH)	0.185	0.175	0.176	0.18	0.004
10	P.E. of *F. montana*(Me-OH)	0.38	0.399	0.34	0.37	0.025
11	P.E. of *F. jangomas* (H_2O)	0.1	0.103	0.105	0.10	0.002
12	P.E. of *F. indica* (H_2O)	0.083	0.079	0.084	0.08	0.002
13	P.E. of *F. montana* (H_2O)	0.169	0.163	0.174	0.17	0.004

Table 6. Comparative data of quantitative analysis of the three selected *Flacourtia spp.*

Sr. No.	Sample	Total Phenolic Content (%w/w)	Total Tannin Content (%w/w)	Total Flavonoid Content (%w/w)
1	*Flacourtia jangomas* (Me-OH)	908.39 ± 0.018	902.66 ± 0.006	420 ± 0.020
2	*Flacourtia jangomas* (H_2O)	794.84 ± 0.044	883.54 ± 0.004	82.01 ± 0.002
3	*Flacourtia indica* (Me-OH)	743.80 ± 0.056	877.86 ± 0.011	240.35 ± 0.004
4	*Flacourtia indica* (H_2O)	678.30 ± 0.020	863.68 ± 0.012	38.96 ± 0.002
5	*Flacourtia montana* (Me-OH)	911.51 ± 0.005	899.85 ± 0.004	645.21 ± 0.025
6	*Flacourtia montana* (H_2O)	874.00 ± 0.031	897.73 ± 0.011	216.67 ± 0.004

n=3, Mean±SD

Table 7. Functional Groups estimated from the FTIR analysis for the AuNPs of three *Flacourtia* leaf extracts.

Sr. No.	I.R. Stretching seen in the three plants	Functional Groups present in the AuNPs of *Flacourtia* leaf extract	Absorption Frequency Region
1	O-H	Hydroxyl compounds	3400-3600
2	C=C	Alkene	1600
3	N-H	Amide	1400

4.3 Biosynthesis of Gold Nanoparticles

The green synthesis of AuNPs was determined primarily by the color change to purplish-ruby red color after adding chloroauric acid to the leaf extracts of the *Flacourtia* spp. **(Plate 7)**. These solutions were then subjected to further physical characterization techniques.

4.3.1 UV–vis analysis of gold nanoparticles

UV–visible spectrophotometry is a critical technique to determine the morphology and stability of nanoparticles. The formation of gold nanoparticles by the cell-free filtrate was observed with a change of color from yellow to ruby-red (Shankar *et al*., 2004), which is the characteristic of gold nanoparticle formation due to the excitation of surface plasmon vibrations in gold nanoparticles. It is well known that metal nanoparticles' optical properties strongly depend on their size and shape. According to the Mie theory, small gold nanoparticles exhibit only one surface plasmon resonance (SPR) absorption band, whereas anisotropic particles show two or three SPR bands **(Figure 3)** showing UV–vis spectra of the aqueous chloroauric acid. In the case of gold ion reduction, the bands corresponding to the SPR occurred at 530-541nm. The solution was highly stable without aggregation for one month after the reaction.

4.3.2 FTIR analysis of gold nanoparticles

In the FT-IR spectrum, the leaf extract of *Flacourtia jagomas*, *Flacourtia indica*, and *Flacourtia montana* showed IR stretching for the functional groups such as hydroxyl compounds(O-H), alkenes, and amides in the peak-ranging between 3400-3600 cm^{-1}, 1600 cm^{-1}, and 1400 cm^{-1} respectively **(Figure 4**, **Table 7)**. Hence the dominant functional groups that entail in bio-reducing of AuNPs are phenolic hydroxyl groups and amino groups that exist herein due to the presence of xanthones and proteins. The

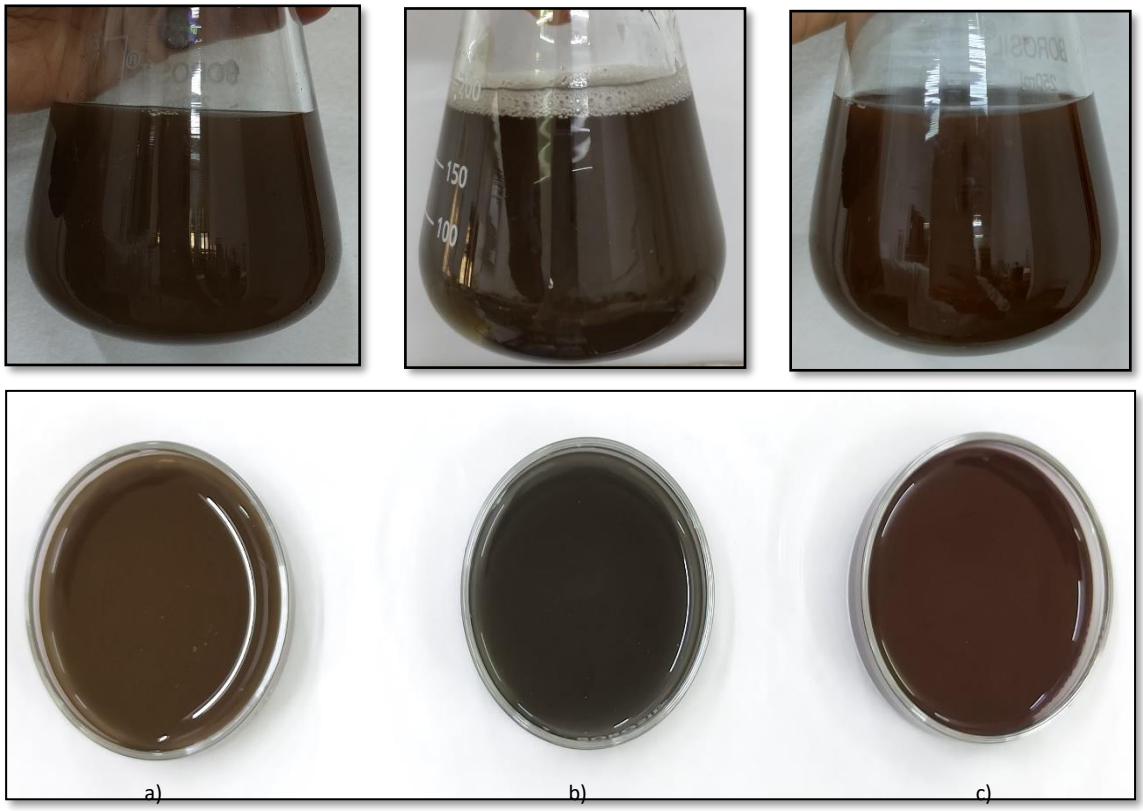


Plate 7. Synthesis of Gold nanoparticles using leaf extracts of a) *F. jangomas.* b) *F. indica* c) *F. montana*

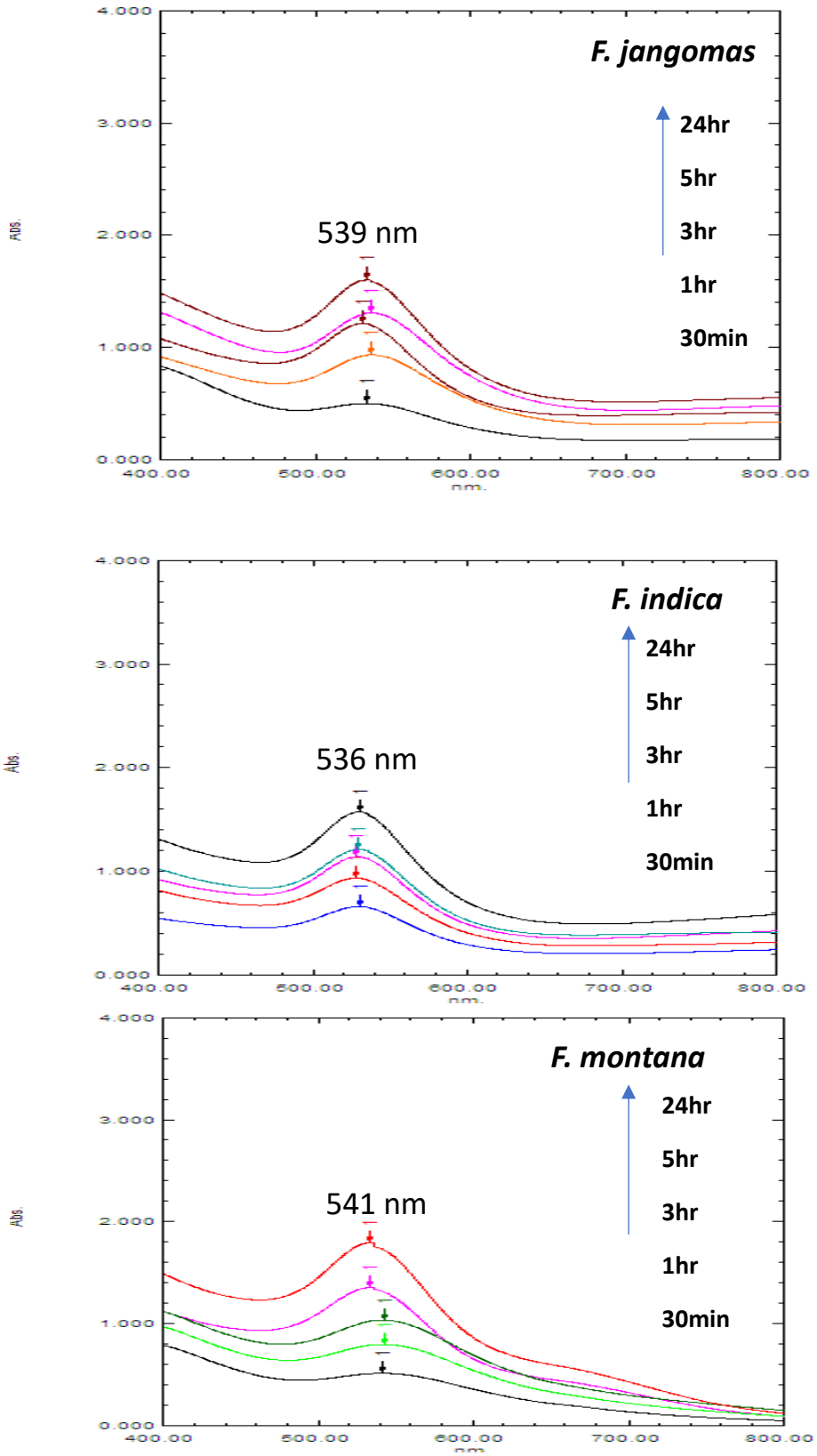


Figure 3. UV–vis spectrum recorded for gold nanoparticles formed using leaf extract *Flacourtia* spp.

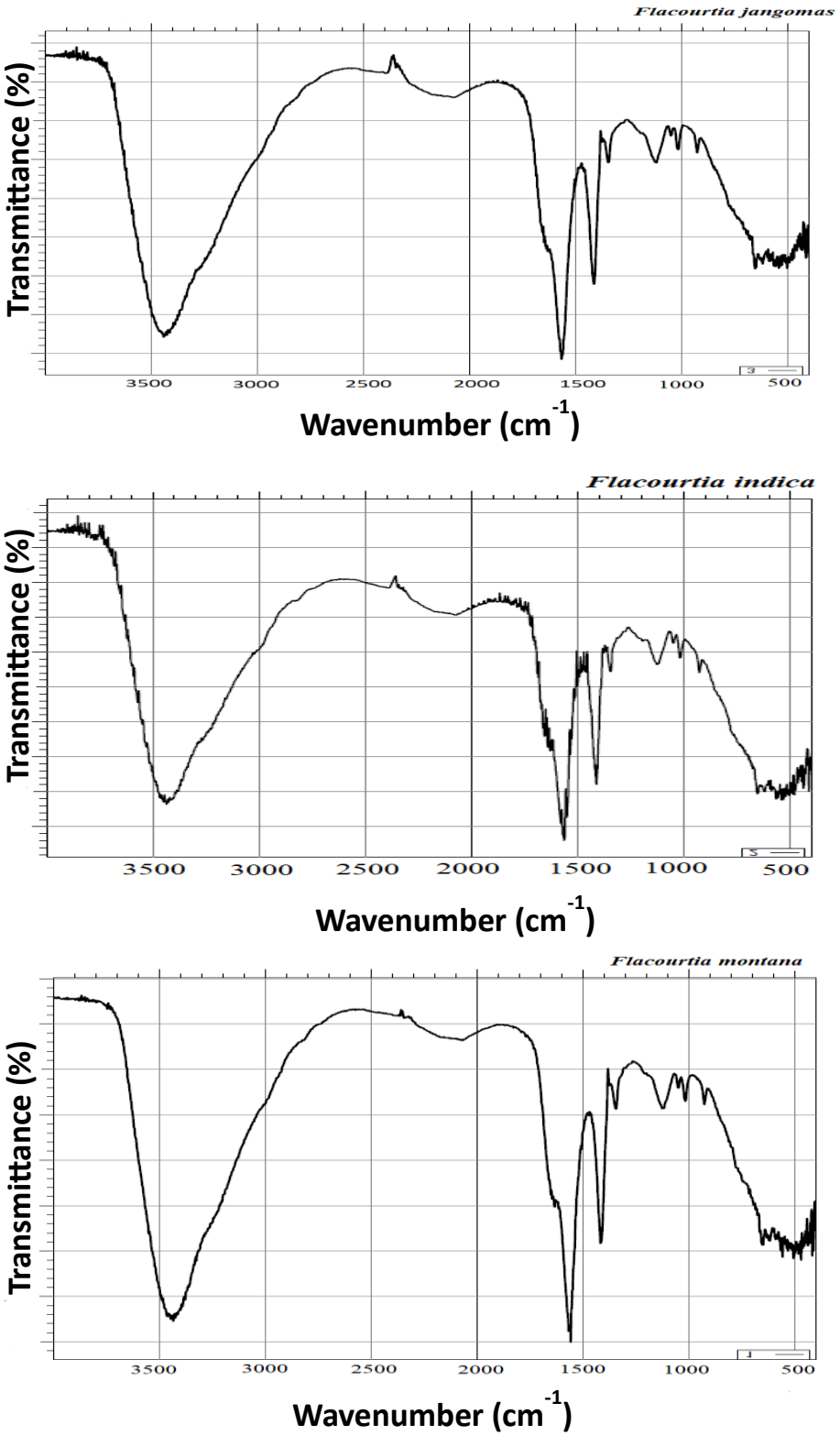


Fig 4. FTIR spectra of gold nanoparticles synthesized from *Flacourtia* spp., leaf extract

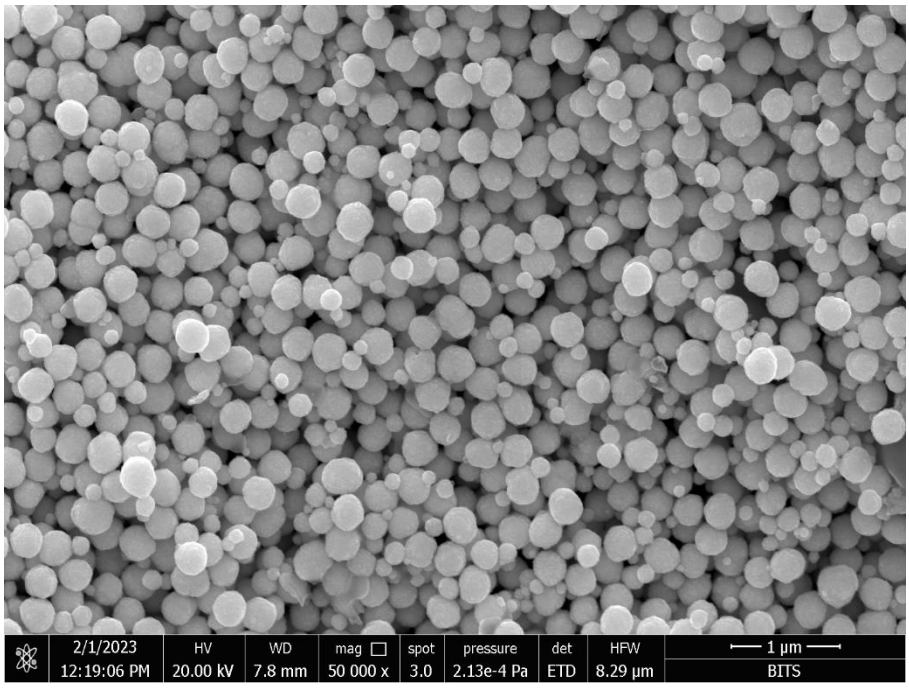
carboxylic groups in the proteins and carboxylic acids adhere to the as-synthesized AuNPs, granting them stability during the synthesis. All these findings are consistent with the literature (Rasheed, 2017) in which hydroxyl and ketone groups and flavonoids are reported to be the bio-reducing agent and carboxylic acid as the capping agent.

4.3.3 Analysis of gold nanoparticles in FE-SEM (EDS)

The obtained morphology revealed that the synthesized gold nanoparticles are almost spherical without other observable nanostructure morphologies, as confirmed by the absorbance spectrum. The gold nanoparticles are spherical, ranging from 53 to 67 nm, as evident by SEM studies **(Plate 8, 9, 10)**. The morphology with the reaction temperature can be discussed concerning the reaction rate. At higher temperatures, most gold ions first form nuclei, and the secondary growth of the particles stops because the reaction rate is very high. The sample was analyzed using EDS techniques to gain further insight into the features of Au nanoparticles. The energy dispersive spectra of the samples obtained from the SEM-EDS analysis show that all three samples prepared by the above route have pure Au phases. The EDS studies of **(Figure 5)** present three peaks between 1 kV and 10 kV. Those maxima are directly related to gold in the tested material. The results indicated that the reaction product was composed of high-purity gold nanoparticles. Additionally, the presence of highly pure Au was confirmed by X-ray diffraction XRD.

4.3.4 X-ray diffraction (XRD) analysis

The X-ray diffraction pattern (XRD) gold nanoparticles are shown in **(Figure 4)** synthesized using leaves of three *Flacourtia* spp., several Bragg reflections with 2θ values of 38.11, 44.25, 64.08, and 77.5, which correspond to the (111), (200), (220), and (311) sets of lattice planes, are observed **(Figure 7)** which are indexed to the face-



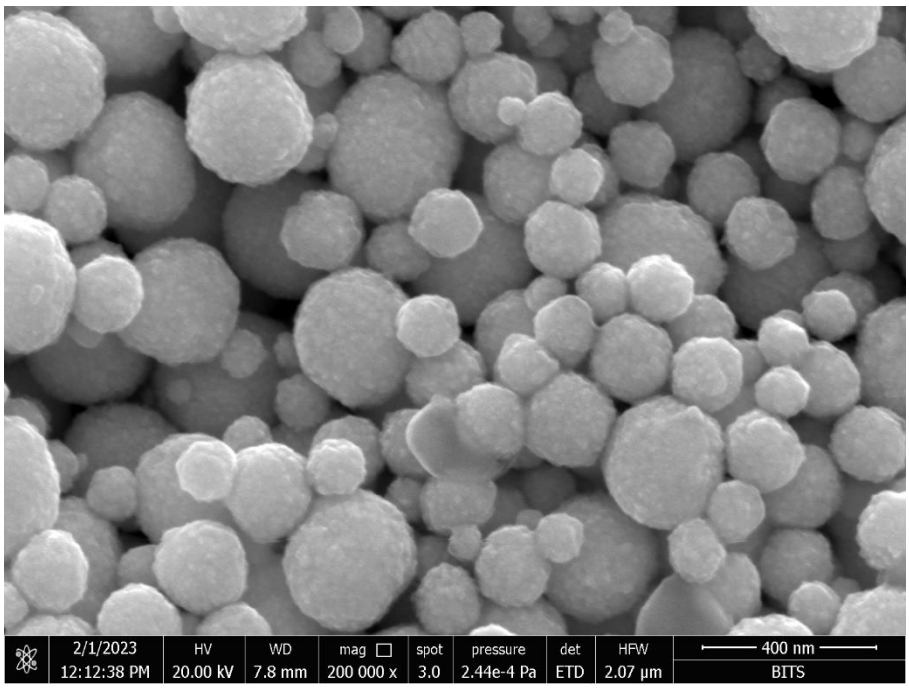


Plate 8. Scanning Electron micrographs of Au nanoparticles synthesized using *Flacourtia jangomas.*

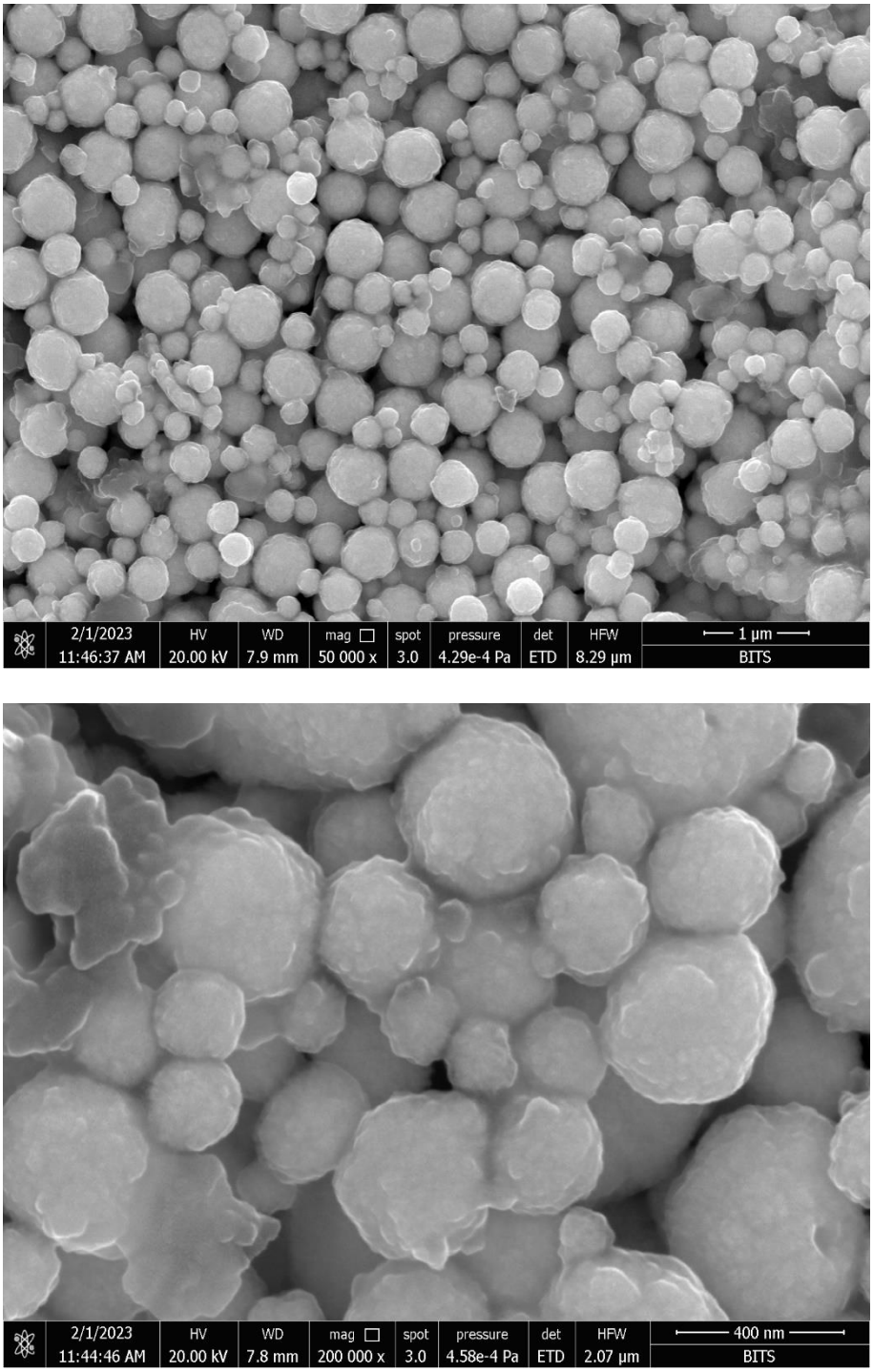


Plate 9. Scanning Electron micrographs of Au nanoparticles synthesized using *Flacourtia indica*

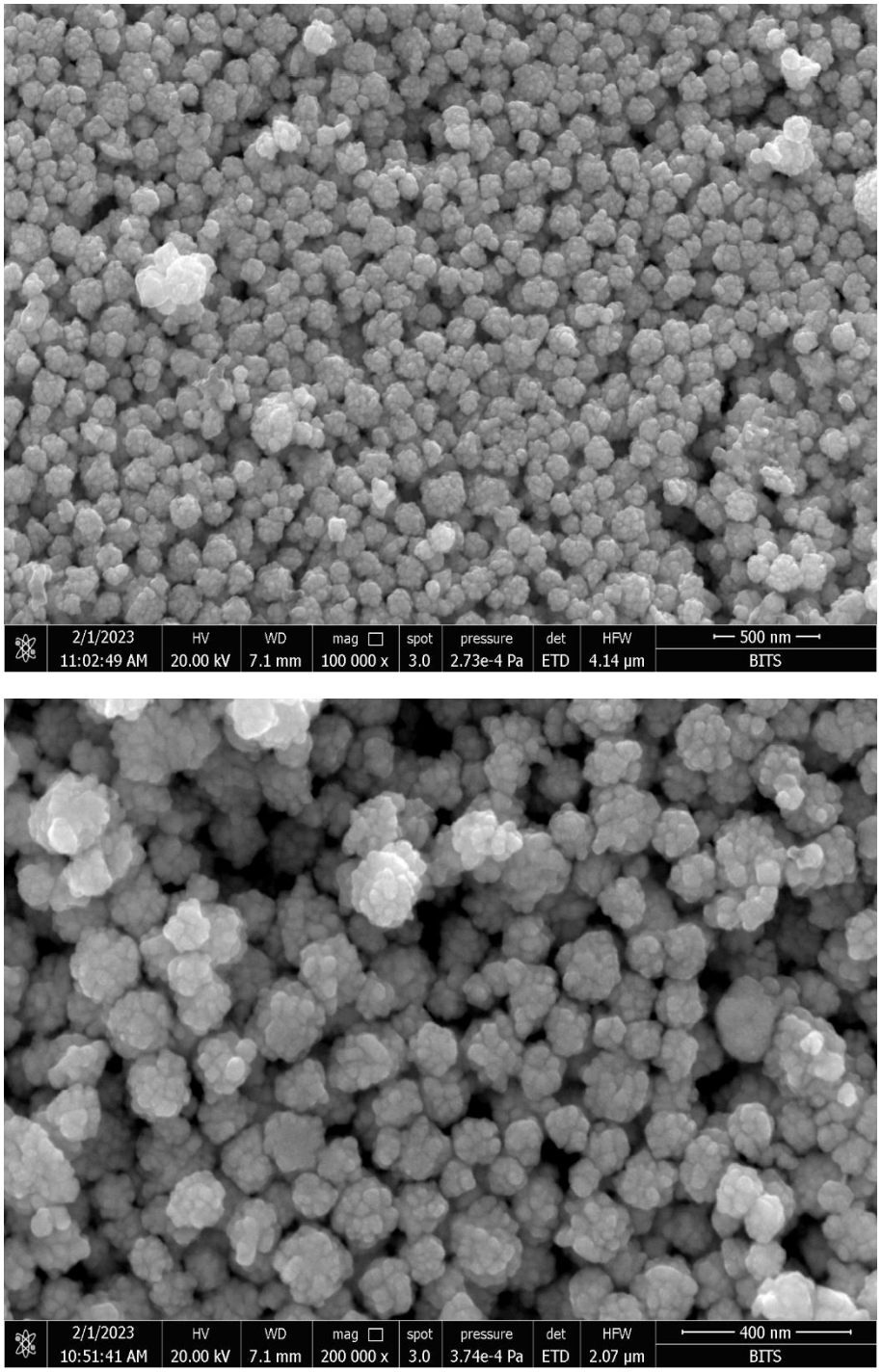
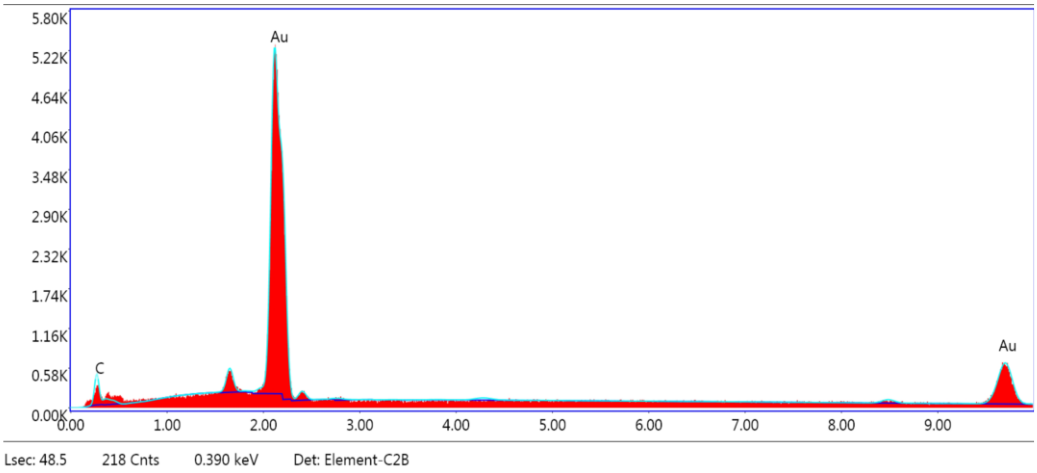
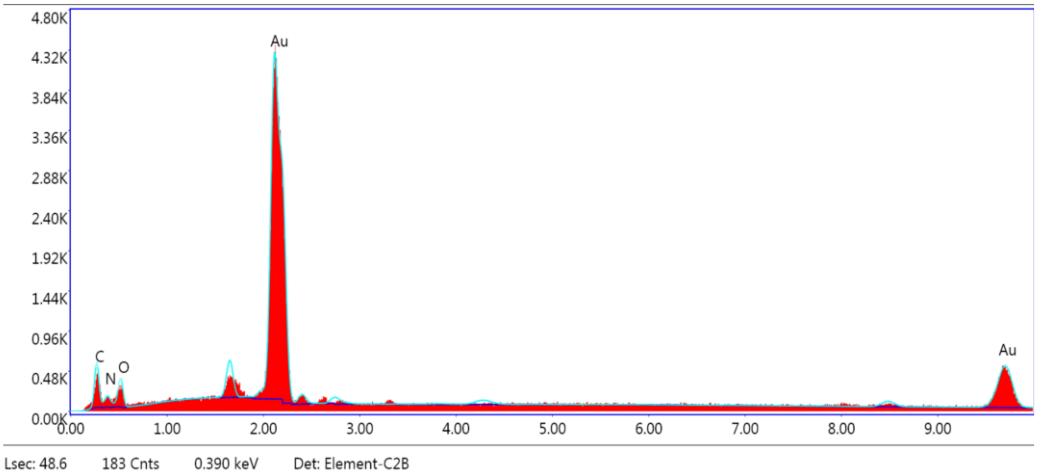


Plate 10. Scanning Electron micrographs of Au nanoparticles synthesized using *Flacourtia montana*

F. jangomas



F. indica



F. montana

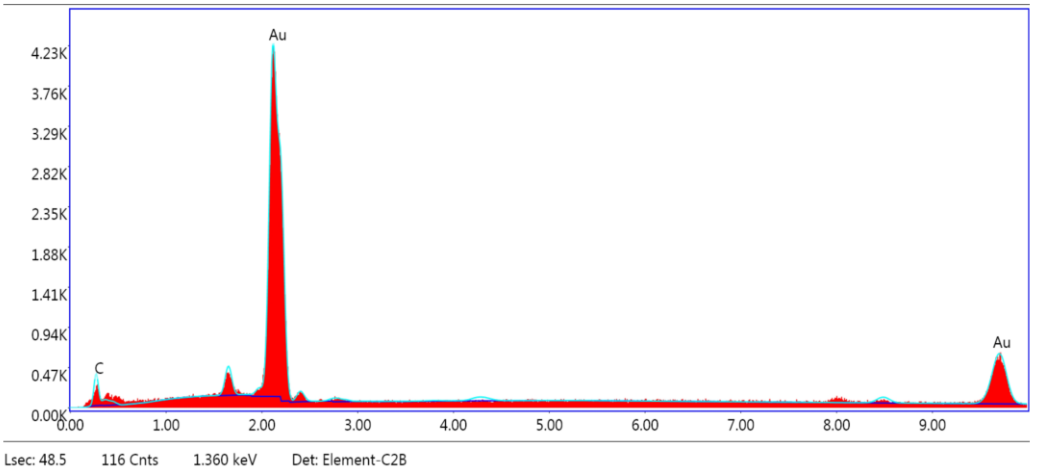


Figure 5. EDS profile of Au nanoparticles synthesized using *Flacourtia* spp.

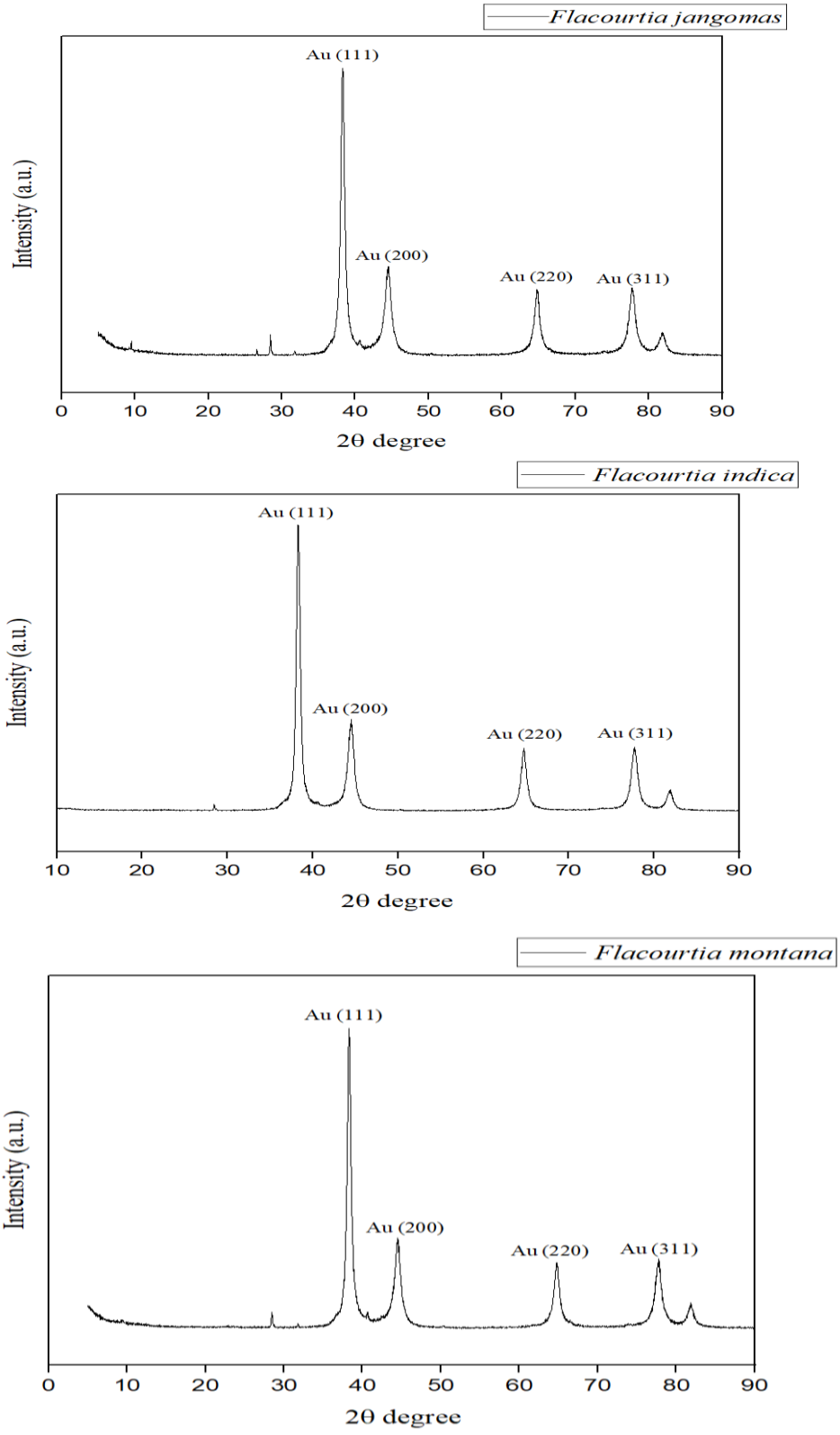


Figure 7. XRD Patterns of Au nanoparticles synthesized using *Flacourtia* spp.

centered cubic structures for gold. The broadening of Bragg's peaks indicates the formation of nanoparticles. A few intense additional and yet unassigned peaks were also noticed in the vicinity of characteristic peaks of gold. These sharp peaks might have resulted from some bio-organic compounds/proteins in the nanoparticle during the synthesis (Gardea-Torresdey *et al*., 2002). The presence of these external peaks, which are unassigned, did not alter the Bragg reflection peaks dedicated to gold, indicating that their presence could also be responsible for the stabilized gold nanoparticles. Thus, the XRD pattern clearly illustrates that the gold nanoparticle synthesized by the green method is crystalline.

4.4 Evaluation of Antimicrobial Assay

The use of antimicrobial agents is critical to the successful treatment of infectious diseases. The use of higher plants and preparations made from them to treat infections is a longstanding practice in large part of the population, especially in developing countries, where there is a dependence on traditional medicine for various ailments (Ahmad and Mohammad 1998). Interest in plants with antimicrobial properties increased because of current antibiotics-associated problems (Emori and Gaynes 1993; Pannuti and Grinbaum 1995). The present study for antimicrobial was carried out for the various concentrations of synthesized AuNPs and plant extracts of *Flacourtia* spp., to check their inhibitory nature towards the selected microbial agents.

4.4.1 Antifungal assay

The study was carried out for antifungal assay using three causative strains *Fusarium* spp., *Aspergillus* spp., and *Penicillium* spp., showed no prominent zones of inhibition. This poor activity may be because the concentrations used did not have a pronounced effect on inhibiting the fungal growth **(Plate 11).**

4.4.2 Antimicrobial assay

The antimicrobial activity showed no prominent inhibition zones for the two leaf extracts, namely methanol and AuNPs extract. The pathogenic strain used was *Escherichia coli* which flourished in the presence of the extract. This poor activity may be because the concentrations used did not have prominent effect for inhibiting the bacterial growth or the strain was unsuitable for the experimental objectives.

Studies showed that, moreover, after entering bacteria, gold nanoparticles reduced adenosine triphosphate (ATP) levels and led to decreased metabolism (Cui, 2012)**.** Pravin *et al*. (2011) studied the antibacterial activity of the crude extract of *F. jangomas* against gram-positive and gram-negative bacteria, which showed good antibacterial activity against *Shigella shiga* and *Bacillus megaterium* and moderate activity against *Bacillus cereus* and poor action against *Escherichia coli* **(Plate 12)**.

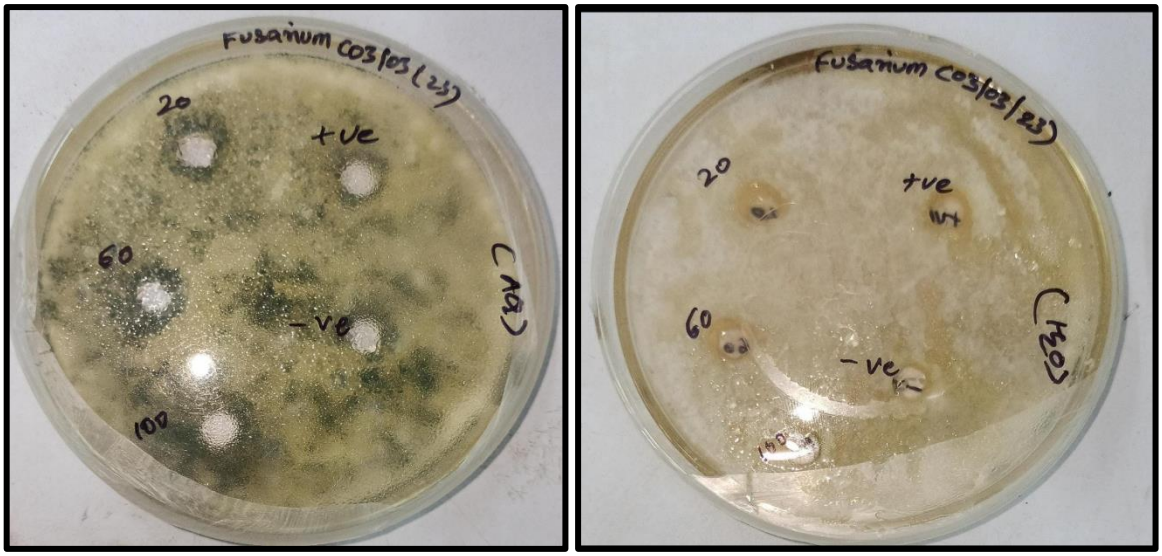


Plate 11. Antifungal assay showing no zone of inhibition using leaf extracts of three *Flacourtia* spp.

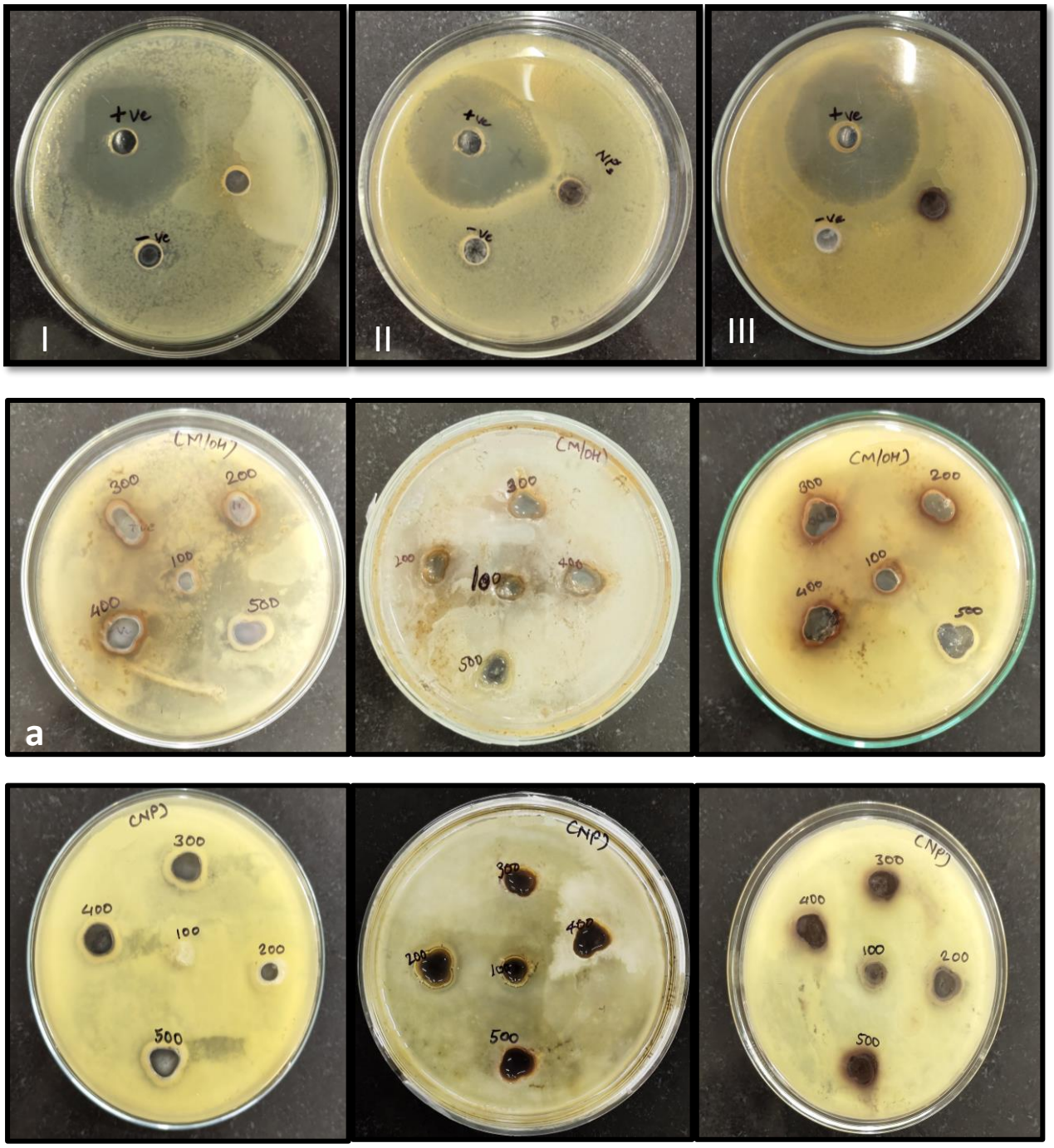


Plate 12. Antibacterial assay showing no zone of inhibition.

I-*Flacourtia jangoma*, II-*Flacourtia indica*, III-*Flacourtia montana* with concentrations of (a) M/OH extract and (b)AuNPs

CHAPTER 6: CONCLUSION

CONCLUSION

In this investigation, qualitative and quantitative phytochemical analysis and green synthesis of gold nanoparticles were carried out using *Flacourtia* species: *Flacourtia jangomas, Flacourtia indica*, and *Flacourtia montana*. Through qualitative analysis, it could be concluded that all three plant species are rich sources of phenols and tannins, while the plants also extracted flavonoids.

A green chemistry approach, an eco-friendly method for synthesizing Au nanoparticles by reducing aqueous $AuCl^{4-}$ ions, was done using the three species of *Flacourtia* leaf broth. UV absorption of colloidal suspension (hydrosol) of Au NPs was used as an easy and quick assay to check the production of NPs. During the reaction period, an increase in absorbance was observed in UV-Vis wavelength, possibly due to the rise in the production of colloidal NPs. The sharp peak at 541nm in the UV-Vis spectrum endorsed the formation of AuNPs. XRD pattern helped identify the face-centered cubic (FCC) structure of the biogenic AuNPs. The SEM analysis showed the formation of stable nanoparticles of spherical morphology for the three samples of *Flacourtia.* The plant species *F. jangomas* was in the range of 16.68-74.34nm, *F. indica* 28.58-68.58nm, and *F. montana* 18.57-45.76nm. The EDS analysis indicated that the reaction product was composed of high-purity gold nanoparticles and was also more evident through the XRD analysis. FTIR spectra showed the presence of different functional groups, such as OH (3400-3600), C=C (1600), and N-H (1400).

The plant extract and synthesized nanoparticles were tested for their antimicrobial activity to check their resistant nature. The antifungal studies performed with the specific experimental concentration showed no prominent zone of inhibition from the three causal agents *Fusarium* spp., *Aspergillus* spp., and *Penicillium* spp., used. Similarly, for the

antibacterial activity, the zone of inhibition was not formed for the plant extract and the AuNP extracts.

From the above studies, it can be concluded that the three selected species of *Flacourtia* are a rich source of various phytoconstituents and thus holds a future in the pharmaceutical industry. This convenient procedure for the biosynthesis of gold nanoparticles has several advantages, such as cost-effectiveness and an eco-friendly approach, and can thus synthesize maximum nanoparticles in a non-hazardous manner. While further studies could be done to test for the antimicrobial activity using different causative agents as the synthesized gold nanoparticles may not be showcasing inhibition for all types of causative agents.

CHAPTER 7: SUMMARY

SUMMARY

The three species of *Flacourtia* from different parts of Goa, namely *F. jangomas, F. indica,* and *F. montana* were used for the phytochemical analysis and green synthesis of gold nanoparticles. For the experimental purpose leaf of every individual plant was used to obtain the extracts for phytochemical analysis and nanoparticle characterization. The presence of phytoconstituents in plant extract was done by following the standard procedures of Raaman (2006), which brought forth the phytochemistry and abundance of the essential constituents in desired study plants. Quantitative estimations gave a comparative idea of the number of polyphenols in the *Flacourtia* spp. UV-Visible spectroscopic analysis showed the peaks that might represent phenols and tannins in the samples.

Green synthesis of gold nanoparticles was confirmed primarily by the color change to ruby red-purplish color noticed upon adding chloroauric acid into the leaf extracts of the three test plants *F. jangomas, F. indica,* and *F. montana.* The UV-Vis spectrum sharp peak at 541nm endorsed the formation of AuNPs. Further characterizations using FE-SEM gave an idea of the spherical morphological nature of the synthesized nanoparticles, and the EDS analysis showed the presence of high-purity gold nanoparticles synthesized. This was further confirmed through the XRD analysis data showing the significant gold percentage in the synthesized nanoparticles. FTIR analysis was done to check the various functional groups in the nanoparticle extracts. Thus, the characterizations gave an idea of the gold nanoparticles synthesized in our sample extract.

From the antimicrobial activity performed, prominent inhibition zones were not observed for any of the nanoparticle leaf extracts and was concluded that it could be due

to the lesser or no resistance effect of the concentrations of extract used towards the different fungal and bacterial causative agents used. Thus, further studies may be carried out to test the antimicrobial activity of varied concentrations of synthesized gold nanoparticles.

Compared to the chemically synthesized NPs, characteristics of plant-mediated synthesis are still relatively unpredictable; however, the existing reports and knowledge on the subject are narrowing the gap between known and unknown (Rani, 2023). It was noticed through various literatures that numerous chemical, physical and biological synthetic methods have been used in the production of metal nanoparticles. Most of these methods are still nascent, and the significant problems experienced are the stability and aggregation of nanoparticles, morphology, size, and size distribution. Moreover, separating produced nanoparticles for further applications remains an important issue. Studies have shown that the metal nanoparticles produced by plants are more stable than those produced by other organisms. Plants (especially plant extracts) can reduce metal ions faster than fungi or bacteria. Furthermore, plant extracts are better than plant biomass or living plants for using easy and safe green methods in scale-up and industrial production of well-dispersed metal nanoparticles (Vidhya, 2016).

CHAPTER 8: REFERENCES

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Qualitative and Quantitative Phytochemical analysis of *Flacourtia jangomas* (Lour.) Raeusch. and *Flacourtia indica (Burm.f.) Merr.*

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Flacourtia jangomas

INTRODUCTION

Flacourtia jangomas (Lour.) Raeusch., the Indian coffee plum or scram berry, and *Flacourtia indica* (Burm.f.) Merr., governor's plum, Madagascar plum, or Indian plum are small deciduous trees belonging to the family Salicaceae. The trees serve as a raw material due to their potential to fulfil several purposes in research, medicine, culinary, etc. Both species contain various secondary metabolites with great potential, which protect the plant cells from environmental hazards such as pollution, stress, drought, harmful UV radiation, and pathogenic attack. The objective of the present study was to evaluate the phytochemical constitution by qualitative and quantitative analysis using methanolic extract of dried leaves of species *F. jangomas* and *F. indica.*

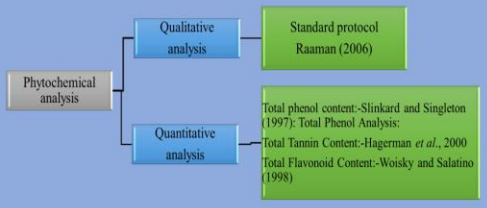
Flacourtia indica

MATERIALS & METHODS

I. Collection of plant sample

- Plant samples were collected from the sites, washed properly and shade dried for 2 weeks .
- The dried leaves were ground to fine powder and 10gm of it was subjected to Soxhlet extraction using methanol as the extracting solvent.
- This was followed by rotary evaporation to obtain solvent free extract.
- The solvent free extract was subjected to qualitative and quantitative phytochemical analysis

II. Phytochemical Analysis



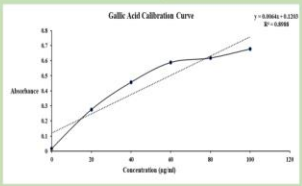
RESULTS & DISCUSSION

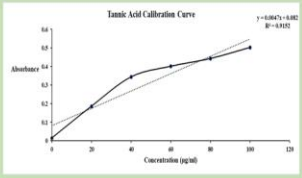
Qualitative analysis

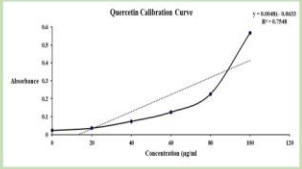
Sr. No.	Phytochemical Test	Methanol Extract	
		Flacourtia jangomas	*Flacourtia indica*
1	Alkaloids		
a	Hager's Reagent	+	+
b	Wagner's Reagent	+	+
2	Carbohydrates & Glycosides		
a	Benedict's test	+	+
b	Barfoed's test	+	+
c	Legals Test	+	+
3	Proteins & Amino acids		
a	Ninhydrin Reagent	-	-
b	Biuret Reagent	+	+
4	Phytosteroids		
	Liebermann-Burchard's test	+	+
5	Fixed oils & Fats		
	Saponification test	-	-
6	Phenolic compounds & Flavonoids		
a	Lead acetate	++	++
b	Ferric chloride test	++	++
7	Gums & Mucilages		
	Alcohol 95% test	-	-

+: Positive; -: Negative; ++: strongly Positive

Quantitative analysis







SAMPLE	Total Phenol Content	Total Tannin Content	Total Flavonoid Content
Methanol Extract	(%w/w)	(%w/w)	(%w/w)
	Mean ± S.D.	Mean ± S.D.	Mean ± S.D.
Flacourtia jangomas	908.39 ± 0.018	902.66 ± 0.006	420 ± 0.020
Flacourtia indica	743.80 ± 0.056	877.86 ± 0.011	240.35 ± 0.004

CONCLUSION

Further studies on different plant parts of the selected species could yield promising results in the field of pharmaceutics, and through this preliminary analysis, we can conclude that the *Flacourtia* spp. could be used as an effective dietary phenol.

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Poster Presentation for National conference on "**Recent Trends in Plant Sciences and Biotechnology**" held during 3rd & 4th November 2022.

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National Conference on Recent Trends in Plant Sciences & Biotechnology

Certificate

This is to certify that Dr./Mr/Ms ✓ Veda Dessai has attended/ ~~Delivered~~ ~~Lecture~~/ Presented a paper (~~Oral~~/✓ Poster) entitled "Qualitative and quantitative phytochemical analysis of Flacourtia jangomas and Flacourtia indica" in the National Conference on Recent Trends in Plant Sciences & Biotechnology, held during 3rd & 4th November 2022, at the School of Biological Sciences and Biotechnology (Botany), Goa University, Goa

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