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Phytochemical analysis and biological activity studies of methanolic extract of *Tabebuia rosea* seeds

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Abstract
The current study was undertaken to assess the phytochemical and antimicrobial properties of the methanolic extract of *Tabebuia rosea*. The Soxhlet extract of seeds, when subjected to preliminary phytochemical screening, showed the presence of Essential oils, Terpenoids, Steroids, Flavonoids, Tannins, Coumarins, Cardioglycosides, Phenols, and Flavons. The methanolic extract and extracted oil were tested for their antimicrobial activity against *Escherichia coli*, *Staphylococcus aureus*, *Candida albicans* and *Aspergillus niger* by Agar Well Diffusion method under in-vitro condition. The potency against microbes was observed in the seed extract and the oil extract of *Tabebuia rosea*.

Keywords: *Tabebuia rosea*, preliminary phytochemical screening, biological activity studies, antioxidant activity

1. Introduction
Plants synthesise a significantly high number of assorted bioactive compounds. A high portion of phytochemicals, which have a potential for protecting against free radical damage, accumulate in vegetables and fruits. Free radicals that may damage cells in the body can be overcome by the presence of an antioxidant [1]. The phytoconstituents which are phenols, anthraquinones, alkaloids, glycosides, flavonoids and saponins add antibiotic values to the plants. From these phytoconstituents, saponins have been reported to exhibit hemolysis, foaming action, anti-inflammatory, anti-fungal, molluscicidal and fungistatic properties [2].
In the search for plant-products with potential bioactivity, studies have shown that some species belonging to the Bignoniaceae family have anti-inflammatory, antimicrobial, and anti-tumoral potential, due to its empirical use in rural Colombia, Bolivia, Brazil, and other Latin American countries. Within this family, *Tabebuia rosea* (Bertol.) DC. has been traditionally used for the treatment of skin conditions such as pruritus and infections linked with fungi and yeast in the Northern Coast of Colombia [3].
Reaching a height of 30m, with a flaky bark surrounding its 1m trunk, the *T.rosea* is a tree characterised by its bell-shaped ornate purple and pink flowers[4]. As only a few studies have evaluated the biological activity of *T. rosea* extracts, the main objective has been in determining the antioxidant, anti-inflammatory, and antiproliferative potential of the leaf and inner bark extracts obtained from it [5]. This study focuses on the phytochemical screening and assessment of the biological activities, such as antimicrobial and antioxidant properties of the *T. rosea* seed extract.

2. Materials and Methods
Preparation of extract [6]: The dried and powdered seeds were extracted using methanol as a solvent with Soxhlet extractor for 12 hours in a hot air oven at 60 ºC. The extract was filtered and then concentrated to dryness, and transferred into a container for further analysis.

2.1 Qualitative phytochemical screening of *Tabebuia rosea* seed extract
The methanolic extract was subjected to several tests for the presence of various bioactive compounds, such as essential/volatile oils, tannins, steroids, cardioglycosides, terpenoids, chalcones, flavonoids, coumarins, starches, anthocyanins, phlobatannins, phenols, carotenoids, free and combined anthraquinones, flavanones, saponins, and emodins, by the tests described

by Ajayi *et al.*, 2011 [2], Waza *et al.*, 2015 [6], Dubey *et al.*, 2014 [7], Joy *et al.*, 2014 [8], Auxilia *et al.*, 2013 [9], and Purewal *et al.*, 2014 [10].

2.2 Biological activity Studies

2.2.1 Determination of antimicrobial activity: The extent to which the sample can inhibit the growth of the microbes under in-vitro conditions is measured in this experiment.

2.2.1.1 Microorganisms

Microbial cultures were acquired from the Microbial Type Culture Collection (MTCC), Institute of Microbial Technology (IMTECH), Pune, India. The cultures used for the experiments were *Escherichia coli* (MTCC-40), *Staphylococcus aureus* (MTCC-7443), *Candida albicans* (MTCC-183), and *Aspergillus niger* (MTCC-1344). Bacterial and fungal stock cultures were maintained on nutrient agar and Sabouraud's dextrose agar slants respectively, at 4 ºC for periodic sub-culturing.

2.2.1.2 Determination of Antibacterial activity

The Agar-Well Diffusion Assay, as described by Ajani *et al.*, 2016[11], was used to determine the extent of growth inhibition of bacteria by the given sample. The test bacteria species used were *Escherichia coli* and *Staphylococcus aureus*. Streptomycin was used as the positive control and methanol as the negative control.

2.2.1.3 Determination of Antifungal activity

A fungal suspension was used to surface inoculate a freshly prepared potato dextrose agar in petri dishes [12]. The organisms used for testing the antifungal activity were *Aspergillus niger* and *Candida albicans*. Fluconazole was used as the positive control, while methanol was the negative control.

2.2.2 Determination of Antioxidant activity

Free radical scavenging capacity by DPPH radical assay 2.2'-diphenyl-1-picrylhydrazyl)

The estimation of free radical scavenging activity of crude extracts of *T. rosea* was carried out as described by Alabri *et al.*, 2014 [13]. Radical scavenging activity of the tested crude extract samples was estimated as an inhibition percentage and was calculated.

The formula for the measurement of radical scavenging activity in percentage is as follows:

$$\%\ \text{inhibition} = \text{A control} - \text{A extract}/\ \text{A control} \times 100$$

Where, A is the absorbance measured under UV-spectroscopy.

ABTS (2, 2'-azino-bis (3-ethyl benzthiazoline-6-sulphonic acid) radical scavenging activity

The total antioxidant activity of the seed extract was measured by the ABTS+ radical cation decolourisation assay and was carried out as described by Siddhuraju, 2006 [14].

3. Results and Discussion

3.1 Phytochemical analysis

The screening for the phytochemical components of the crude methanolic extract of *T. rosea* was performed using generally accepted laboratory methods for qualitative determinations. The results are as demonstrated in Table 1.

The tests indicated the presence of essential oils, terpenoids, steroids, flavonoids, tannins, coumarins, cardioglycosides, phenols, and flavons. The presence of an oily substance in the extract was determined to be positive by the Spot Test. These phytochemical compounds are known to play an important role in the bioactivity of medicinal plants. The therapeutic values of these plants lie in their phytochemical compounds, and as such produce definite physiological actions on the human body [15]. However, the tests also indicated the absence of phytochemicals like starch, anthocyanins, phlobatannins, carotenoids, free as well as combined anthraquinones, chalcones, saponins, and emodins.

Table 1: Phytoconstituents of *T. rosea* seed extract.

Name of Phytochemical	Result	Name of Phytochemical	Result
Anthocyanins	Absent	Flavons	Present
Cardioglycosides	Present	Free Anthraquinones	Absent
Carotenoids	Absent	Phenols	Present
Chalcones	Absent	Phlobatannins	Absent
Combined Anthraquinones	Absent	Saponins	Absent
Coumarins	Present	Starch	Absent
Emodin	Absent	Steroids	Present
Essential oil/ Volatile oil	Present	Tannins	Present
Flavonoids	Present	Terpenoids	Present

Flavonoids of *T. rosea* are shown to benefit health due to their ability to scavenge for hydroxyl and superoxide-anion radicals [15]. They also exhibit anti-inflammatory, anti-angiogenic, anti-allergic, analgesic, and antioxidant properties [16]. Besides flavonoids, glycosides, tannins and alkaloids, have been well documented for their hypoglycemic activity [17, 18]. Carotenoids and flavonoids have been known for their anti-metastatic activity, along with their inhibitory effects on tumours, and even lung cancers [19].

Tannins exert antimicrobial mechanisms by iron deprivation, hydrogen bonding or specific interactions with vital proteins such as enzymes in microbial cells [20]. Herbs that have tannins as their component are astringents, and are used for the treatment of intestinal disorders, such as diarrhoea and dysentery [21]. Tannins are used in the dye industry, as caustics for cationic dyes (tannin dyes), and also in the production of inks (iron gallate ink). In the food industry, tannins are used to clarify wine, beer, and fruit juices. It is also used as a coagulant in rubber production.

Glyocosides are naturally-occurring cardioactive drugs used in the treatment of congestive heart failure and cardiac arrhythmia. Coumarins, flavones, carotenoids and phenols are reported as inhibitors of procarcinogen activation, drug-binding inducers of carcinogens, and inhibitors of tumorigenesis [22].

Terpenoids are known for their biological activity as neuropharmacological agents, antioxidants and chemotherapy. Terpenoids are believed to be anticarcinogenic (e.g. perilla alcohol, taxol), antimalarial (e.g. artemisinin), anti-ulcer, antimicrobial, and diuretic (e.g. glycyrrhizin). Furthermore, terpenes play a major role as signal compounds and growth regulators (phytohormones) of plants; they function as phytoalexins in direct defence, or as signals in the indirect defence responses [22].

3.2 Biological activity Studies

3.2.1 Determination of antimicrobial activity

The result of in-vitro antimicrobial screening of the seed oil sample against the two bacteria and two fungi was as presented in Table 2, wherein *Streptomycin* and *Fluconazole* were used as the clinical reference for the antibacterial and antifungal activity evaluation, respectively. The organisms taken were *Escherichia coli, Staphylococcus aureus, Candida albicans* and *Aspergillus niger.*

Table 2: Inhibition zone of Seed extract and Seed Oil for different microorganisms.

Microorganisms	Inhibition zone diameter (mm)				
	Seed extract	**Seed Oil**	***Streptomycin***	***Fluconazole***	**Methanol**
Escherichia coli	No inhibition	No inhibition	18	-	No inhibition
Staphylococcus aureus	No inhibition	No inhibition	45	-	No inhibition
Candida albicans	No inhibition	No inhibition	-	31	No inhibition
Aspergillus niger	No inhibition	No inhibition	-	34	No inhibition

The antimicrobial activity of the methanolic seed extract and the oil extract did not show any inhibition against both the bacteria and fungi. Therefore, low potency of the sample was observed in the seed extract and the oil extract of *T. rosea*. The results obtained are shown in figures 1 to 4.

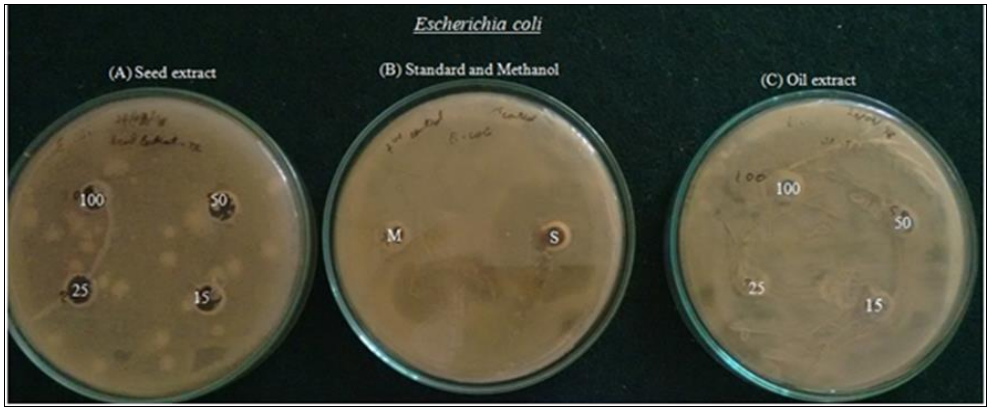

Fig 1: *Escherichia coli* (A) Test for inhibition zone of seed extract. (B) Test for inhibition zone of Standard and Methanol. (C) Test for inhibition zone of Oil extract.

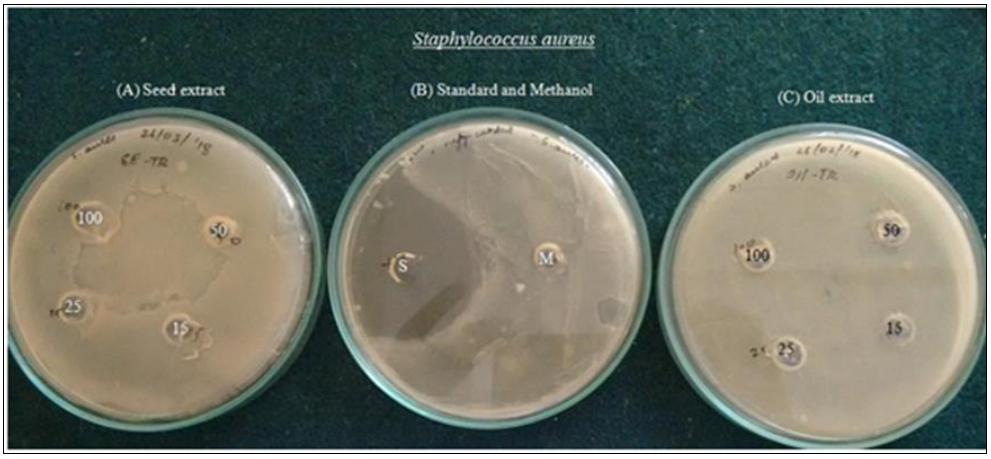

Fig 2: *Staphylococcus aureus* (A) Test for inhibition zone of seed extract. (B) Test for inhibition zone of Standard and Methanol. (C) Test for inhibition zone of Oil extract.

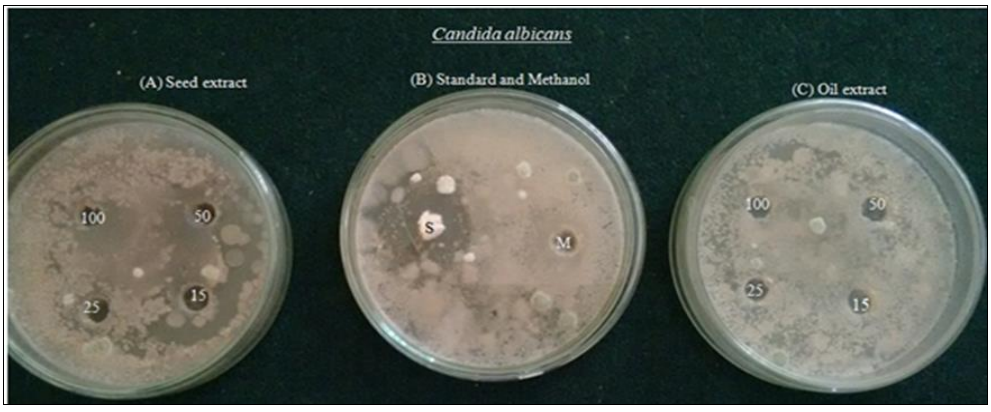

Fig 3: *Candida albicans* (A) Test for inhibition zone of seed extract. (B) Test for inhibition zone of Standard and Methanol. (C) Test for inhibition zone of Oil extract.

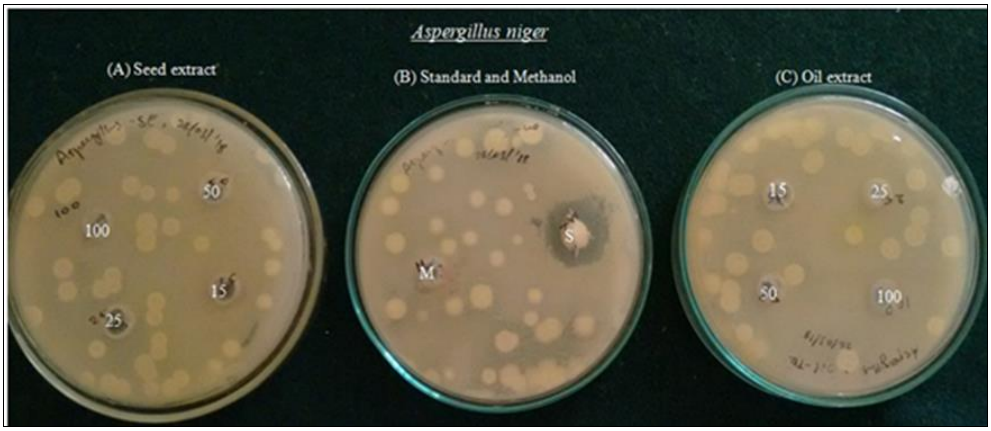

Fig 4: *Aspergillus niger* (A) Test for inhibition zone of seed extract. (B) Test for inhibition zone of Standard and Methanol. (C) Test for inhibition zone of Oil extract.

3.2.2 Determination of Antioxidant activity

An antioxidant is any substance that when present at relatively low concentrations, compared with those of oxidisable substrate, significantly delays or inhibits oxidation of that substrate. In other words, antioxidants are those that scavenge reactive oxygen species (ROS) and reactive nitrogen species (RNS) products including free radicals. These highly reactive free radicals and oxygen species produced during the cellular metabolism are essential for cell signalling, apoptosis gene expression and ion transportation. However, the excess amounts in the body can attack biological macromolecules such as proteins, nucleic acids, lipids and DNA, oxidize them, and cause cell-ageing, cancer and other degenerative diseases.

In the present investigation, DPPH assay and ABTS assay were carried on for the methanol extract of the seed and the oil extract of the *Tabebuia rosea* seeds.

The percentage inhibition by DPPH method for 500, 250, 125.5, 65.5 and 35.25 µg/ml of the Oil extract was 18.41 ± 2.39, 16.31 ± 2.64, 13.93 ± 2.54, 13.93 ± 1.98 and 6.30 ± 2.73 respectively. However, the percentage of inhibition of the standard taken was 95.39 ± 1.10.

The percentage inhibition by DPPH method for 500, 250, 125.5, 65.5 and 35.25 µg/ml of the Seed extract was 27.63 ± 0.27, 26.28 ± 0.74, 19.37 ± 2, 17.84 ± 1.38 and 16.23 ± 2.09. The percentage of inhibition of the standard taken was 94.31 ± 0.09. The DPPH assay and ABTS assay results are shown in figures 5 and 6. The percentage inhibition by ABTS method for 500, 250, 125.5, 65.5 and 35.25 µg/ml of the Oil extract was 42.87 ± 0.88, 42.14 ± 0.76, 38.83 ± 2.05, 33.86 ± 0.24 and 28.14 ± 7.25 respectively. However, the percentage of inhibition of the standard taken was 98.54.

The percentage inhibition by ABTS method for 500, 250, 125.5, 65.5 and 35.25 µg/ml of the Seed extract was 49.46 ± 0.30, 44.7 ± 0.82, 40.51 ± 0.34, 35.84 ± 0.50 and 32.43 ± 1.45 respectively. The percentage of inhibition of the standard taken was 97.67 ± 0.15.

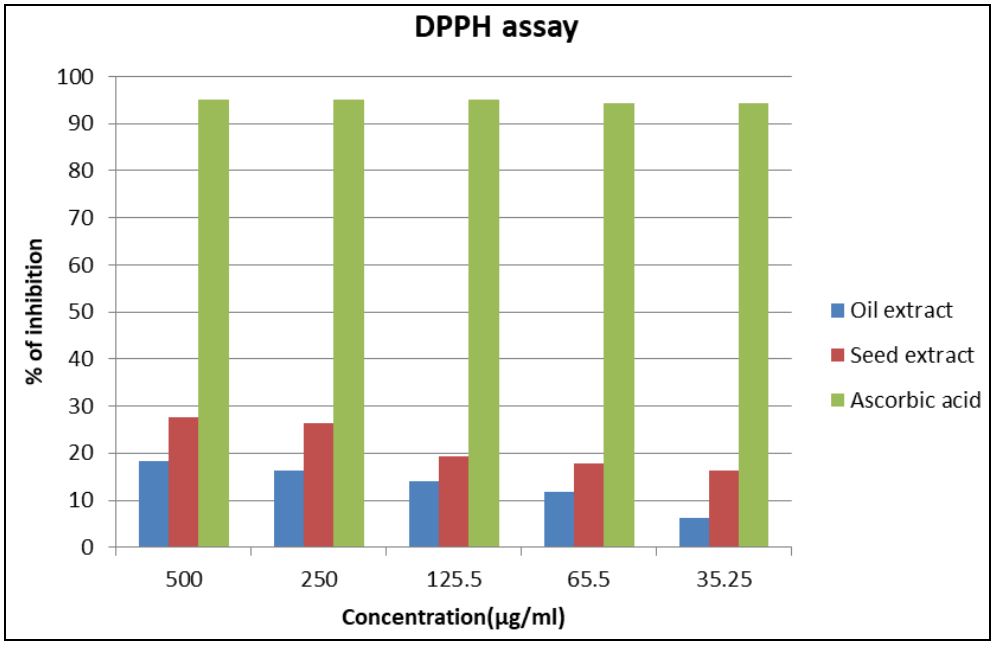

Fig 5: DPPH assay of seed extract and oil.

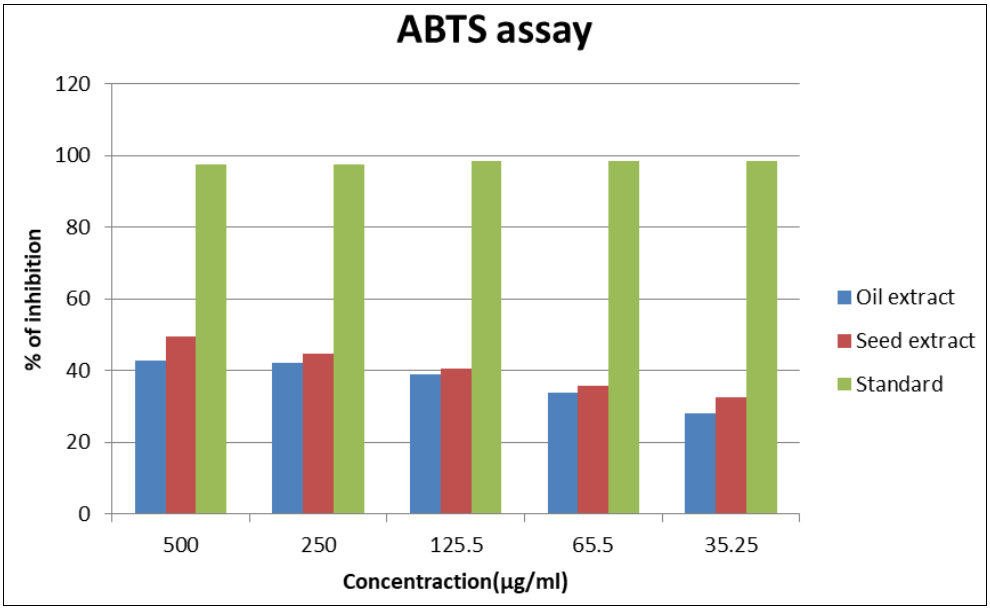

Fig 6: ABTS assay of seed extract and oil.

4. Conclusion

This study adds to the available literature of the biological activity of *Tabebuia rosea*. The results indicate that *T. rosea* extracts show a promising antioxidant, anti-inflammatory, and antiproliferative utility. Further studies are required to isolate the molecules responsible for these activities and elucidate their mechanisms of action.

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