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Antimicrobial Activity of Isolated Flavonoid Fractions from *Drypetes Roxburghii* (Wall.) Huresawa and Its Phytochemical Fingerprinting

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ABSTRACT: The present study investigates the qualitative and quantitative analysis of the major bioactive constituents of different parts like root, stem and leaf of medicinally important plant *Dryptes roxburghii* using seven different solvents. Qualitative study of alkaloids, carbohydrates, glycosides, steroids, flavonoids, coumarins, saponins, fatty acids, tannins, protein and amino acids, gum and mucilage, terpenoids, anthroquinones and phenols showed different types of results in different solvents. Quantitative estimation revealed that phytochemicals are in between the following range alkaloids in between the range (9.5 to 9.6%), flavonoids (18.63 to 21.23%), saponins (11.36 to 5.13%), phenolics (28.36 to 31.1%), tannins (13.16 to 15.38%) and terpenoids (18.8 to 21.56%).

KEY WORDS: *Drypetes roxburghii*, Euphorbiaceae, phytochemicals, flavonoid and antimicrobial.

I. INTRODUCTION

Dryptes roxburghii (Wall.) Huresawa is an esteemed member of Euphorbiaceae (vernacular names- English: Lucky bean tree, Hindi: Putijia, Marathi: Jivan Patravanti, Kannada: Amani Putranjiva). The plant is moderate sized evergreen tree grows up to 13 m in height. Bark is dark grey with horizontal lenticels. Leaves are simple, alternate, dark green, shiny, elliptic-oblong, distantly serrulate, main nerves numerous; Flower are unisexual, Male flowers are very short pedicellate in rounded axillary clusters, female flowers 1-3 in an axil, fruits ellipsoid or rounded drupes and solitaire.

D. roxburghii is distributed in Thailand, Nepal, Bangladesh, India (Western Ghats- in dry zones of South, Central and south Maharashtra Sahyadris), Indochina, Myanmar and Sri Lanka. Traditionally, *D. roxburghii* is used to treat azoospermia, diuretic, catrrah, opthalmopathy, constipation [1] anti-inflammatory, analgesic and antipyretic [2]. The plant is known to treat aphrodisiac, elephantiasis, eye infection, habitual abortion and sterility, laxative [3] and it is used for the treatment of cough, cold and fever [4]. The plant is also used to make toys due to its soft, light and flexible woods. The toy making industries especially artisans of Nirmal toys (Andhra Pradesh) are dependent on this wood for their livelihood [5].

A plant's medicinal value is due to the presence of some chemical substance that produces a physiological action on the Human body and therefore researchers always try to isolate these chemical substances from plants. With the same intent, Garg and Mitra [6] had successfully isolated Roxburghonic acid, and putraflavone from the alcoholic extract of *D. roxburghii* leaves. *D. roxburghii* is acknowledged for its medicinal properties so it's important to reveal other medicinal and phytochemical elements. The phytochemical compound contains polar and non polar functional groups and therefore their solubility varies in different solvents. Many researchers have already carried out the preliminary phytochemical study of *D. roxburghii* using one or two solvents. In the present study, knowing the importance of

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solubility of phytochemicals in their estimation process, we have selected 7 solvents from polar to non-polar range. In the present investigation, the antimicrobial activity of isolated flavonoid fractions was also assayed.

II. MATERIAL AND METHODS

Collection of Plant material

D. roxburghii was collected from Sirsi, Western Ghats of Karnataka and now plant is being maintained in the Department of Molecular Biology, Bangalore University, Bangalore.

Preparation of plant extract

Leaf, stem and roots were collected from *D. roxburghii*. They were dried for one week at room temperature (in shade). Dried plant parts were grinded in a blender to fine particles. Crude plant extract was prepared by soxhlet extraction method. Range of seven polar to non-polar solvent was selected for the study, namely methanol, ethanol, water, chloroform, petroleum ether, hexane and acetone. 20 gm of dried fine grinded powder was uniformly packed into thimble and phytochemicals were extracted with 250 mL of seven mentioned solvents separately. The extraction was carried out for 24 hours. Later extract was concentrated by keeping it on hot plate at 30 to 40°C and stored at 4°C for further research.

Phytochemical screening

Qualitative study

Phytochemical qualitative study of plant extracts of different parts (root, stem and leaves) of *D. roxburghii* was carried out. For the given study, alkaloids, carbohydrates, glycosides, steroids, flavonoids, coumarins, saponins, fatty acids, tannins, protein and amino acids, gum and mucilage, terpenoids, anthroquinones and phenols were estimated.

Test for alkaloids [7]

The plant extracts was dissolved in chloroform and the solution was extracted with dil. H_2SO_4 and acid layer taken and tested for presence of alkaloids.

Dragendroff's test

To 2 mL of acid layer of test solution, 2 mL of Dragendroff's reagent (potassium bismuth iodide solution) and 2mL of dil. HCl were added. An orange-red precipitate indicated the presence of alkaloids.

Mayer's test

To the 1 mL of acid layer of test solution, 1 mL of Mayer's reagent (potassium mercuric iodide solution) was added. Whitish or cream colored precipitate indicated the presence of alkaloids.

Wagner's test

To the 1 mL of acid layer of test solution, 2mL of Wagner's reagent (iodine in potassium iodide) was added. Reddish brown colored precipitate indicated the presence of alkaloids.

Anthraquinone (Borntrager's test)

About 0.5 g of the test solution was taken into a dry test tube and 5 mL of chloroform was added and shaken for 5 min. The extract was filtered and the filtrate shaken with equal volume of 10% ammonia solution. A pink violet or red color in the ammoniacal layer (lower layer) indicated the presence of anthroquinone [8].

Test for carbohydrates

Molisch's test

To 2 mL of the test solution, 1mL of α-napthol solution was added, concentrated sulphuric acid (H_2SO_4) was poured through the sides of the test tube. Purple or reddish violet color at the junction of the two liquids revealed the presence of carbohydrates.

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Barford's test
To 0.5 mL of test solution, 3 mL of barfoed's solution was added; the tubes were placed in a boiling water bath A rusty or brownish-red color indicated presence of monosaccharides.

Benedicts test
To 0.5 mL of test solution, 3 ml of Benedict's reagent was added. The test tubes were shaken to assure uniform mixing. The tubes were placed in a boiling water bath for 3 minutes. Red or green or yellow ppt. was obtained and showed presence of reducing sugar

Cardiac glycosides (Keller Killiani's test)
1 mL of glacial acetic acid was dissolved in 100 ml of test solution containing one drop of ferric chloride solution. This was then underlayer with 1 mL of concentrated Sulphuric acid. A brown ring obtained at the interface indicated the presence of Cardiac glycosides [8].

Coumarins test
In a test tube, 1 ml of each of the test solution were placed and covered with filter paper moistened with dilute sodium hydroxide (NaOH), then heated on water bath for a few minutes. The filter paper was examined under UV light, yellow fluorescence indicated the presence of coumarins [9]

Fatty acids test:
5 mL of test solution was mixed with 5 mL of ether. This extract was allowed to evaporate on filter paper and dried the filter paper. The transparency on filter paper indicates the presence of fatty acids [10].

Test for Flavonoids
Shinoda's test
About 1 ml of each of the extracts was dissolved with 5 ml of ethanol (98 %). To this a small piece of magnesium foil metal was added, this was followed by drop wise addition of concentrated hydrochloric acid. Intense cherry red colour indicated the presence of flavonones. Orange red colour indicated the presence of flavonols [11].

Lead acetate test
Few drops of lead acetate solution were added to each of the extracts in test tubes. Formation of yellow coloured precipitate indicated the presence of flavonoids [12].

Alkaline reagent test
About 1 ml test solution was treated with few drops of sodium hydroxide solution and observed for intense yellow coloration which disappeared on the addition of dilute HCl [13].

Detection of Gum and Mucilage
The plant extract was dissolved in 10 mL of distilled water and to this; 25 mL of absolute alcohol was added with constant stirring. White or cloudy precipitate indicated the presence of gums and mucilages [14].

Ninhydrin test
Two drops of freshly prepared 0.2% ninhydrin reagent (0.1% solution in n-butanol) was added to the small quantity of extract solution and heat it. Development of blue color reveals the presence of proteins, peptides, or amino acids [15].

Test for Phenols
Ferric chloride test: To 10 mL of alcoholic solution of extract, 2 mL of distilled water followed by drops of 10% aqueous Ferric chloride ($FeCl_3$) solution was added. Formation of blue colour indicates the presence of phenols [10].

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Test for Saponins
About 0.5 mL of the plant extract was shaken with water in a test tube. Frothing, which persist on warming was taking as a preliminary evidence for the presence of saponin. Few drops of olive oil was added to 0.5 g of the extract and vigorously shaken. Formation of soluble emulsion in the extract indicated the presence of saponin [16].

Test for Steroids
Liebermann Burchard Test: To 1 mL of extract, 1mL of glacial acetic acid and 1mL of acetic anhydride and two drops of concentrated sulphuric acid were added. The solution becomes red, then blue and finally bluish green, indicates the presence of steroids [17].

Tannins test
The extract of the sample was treated with 15% ferric chloride test solution. The resultant colour was noted. A blue colour indicated the presence of hydrolyzable tannin or into 10 mL of freshly prepared potassium hydroxide (KOH) in a beaker; 0.5 g of the extract was added and shaken to dissolve. A dirty precipitate observed indicates the presence of tannin [16] & [18].

Test for terpenoids
Salkowski test: To 0.5 mL of each the extract was added to 2 mL of chloroform. 3 mL of concentrated sulphuric acid (H_2SO_4) was carefully added to form a layer. A reddish brown colouration of the interface indicated the presence of terpenoids [18].

Phytochemical quantitative test
The extracts were subjected to quantitative phytochemical tests for plant secondary metabolites such as alkaloids, flavonoids, saponins, phenolics, tannins and terpenoids.

Alkaloid determination [19]
In 250 mL conical flask, 5g of the dried fine powdered plant leaf sample was taken and 200 mL of 10% acetic acid in ethanol was added and covered and allowed to stand for 4 h. This was filtered and the extract was concentrated on a water bath to one-quarter of the original volume. Concentrated ammonium hydroxide was added dropwise to the extract until the precipitation was complete. The whole solution was allowed to settle and the precipitate was collected and washed with dilute ammonium hydroxide and then filtered. The residue is the alkaloid, which was dried and weighed.

Flavonoid determination [20]
10 g of the plant sample was extracted repeatedly with 100 mL of 80% aqueous methanol at room temperature. The whole solution was filtered through whatman filter paper No 42 (125 mm). The filtrate was evaporated into dryness over a water bath and weighed.

Saponin determination [21]
In conical flask, 20 g of dried fine particles plant sample was taken and 100 mL of 20% aqueous ethanol was added. This mixture was heated (55˚C) on water bath for 4 h with continuous stirring. Later the mixture was filtered and the residue re-extracted with another 200 mL of 20% ethanol. This extract was further reduced to 40 mL over hot water bath (90˚C). The concentrated extract was transferred into a 250 mL separating funnel and 20 mL of diethyl ether was added and shaken vigorously. Ether layer was discarded and aqueous layer was collected. This step of purification was repeated. 60 ml of n-butanol was added. The combined n-butanol extracts was washed twice with 10 mL of 5% aqueous sodium chloride. The remaining solution was heated in a waterbath. After evaporation the samples were dried in the oven and weighed.

Determination of tannin content [22]
In 250 mL conical flask, 5 g of powdered plant sample was taken and 50 mL of distilled water was added and shook it vigorously for an hour. Later this solution was filtered into a volumetric flask and 5 mL of this filtrate is pipetted out

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into a test tube. The sample were incubated for 1.5 hours at 20 – 300˚ C and the sample was then filled with distilled water up to mark of 50 mL of the volumetric flask. Tannic acid was used as standard; 0.1g of tannic acid was dissolved in 100 mL of water to form tannic acid solution. Distilled water was used as blank. The absorbance of the samples was measured at 760 nm. The values generated were used to calculate the tannin content.

Determination of total phenols [23] & [24].
The sample was boiled with 50 mL of ether for the extraction of the phenolic component for 15 min. 5 mL of the extract was taken into a 50 mL flask, then 10 mL of distilled water was added. 2 mL of ammonium hydroxide solution and 5 mL of concentrated amyl alcohol were also added and left to react for 30 min for colour development. This was measured at 505 nm. The standard curve was prepared using 0, 50, 100, 150, 200, 250 mg/L solutions of gallic acid in methanol: water (50:50 v/v).

Estimation of total terpenoid content [25]
1 g of sample was taken separately and soaked in alcohol for 24 hrs. Then filtered, the filtrate was extracted with petroleum ether; the ether extract was treated as total terpenoids.

Antimicrobial study
Isolation of flavonoid fractions
Leaves of *D. Roxburghii* were collected, dried for one week at room temperature (in shade) and grinded in a blender to fine powder. Crude plant extract was prepared by soxhlet extraction method. The extract was prepared by using 750 g of the powdered leaf of *D. Roxburghii* using a soxhlet apparatus with methanol for 8 h. The extracted solution was filtered and concentrated in a rotary evaporator under reduced pressure (rotary vacuum flash evaporator).
The crude methanol extract (28 g) obtained was subjected to chromatography (Silica gel 120 mesh, 500 g) with eluted with ethyl acetate–n-hexane (7:3) solvent system. A total of 60 fractions were eluted. A Shinoda test was carried out to confirm the presence of flavonoids. Appearance of pink colour indicated presence of flavonoids. Fractions 1-25 were negative. Small Fractions 26-30, 35 - 40, 50-55 gave positive test. These fractions were pooled to form fraction I, II and III.

Antimicrobial studies
The pure axenic cultures of bacteria and fungi were used to study anti-microbial activity of isolated flavonoid fractions. Bacteria viz. *Escherichia Coli, Salmonella typhimurium and Pseudomonas aeroginosa* and fungi viz. *Apergillus fumigates, Apergillus niger* and *Microporum gypseum* were procured from the Department of Microbiology, Bangalore medical college, Bangalore and were further maintained on nutrient agar slants at 4˚C, in the Department of Molecular Biology Bangalore University, Bangalore. The antimicrobial activity was assayed by Agar well diffusion assay [26]. This technique depends upon the diffusion of test solution i.e. flavonoids fraction I, II and III isolated from leaf of *Dryptes roxburghii* and standard drugs (for bacteria – streptomycin and for fungi – nystatin) from a cavity through the solidified agar layer to an extent such that growth of the added micro organism is inhibited entirely in circular area or zone around the cavity. The observed marked zone of growth of inhibition of bacteria were measured with the help of scale and recorded. This experiment was performed in triplicates in order to confirm the reproducibility.

III. RESULTS

The preliminary phytochemical profile of *D. roxburghii*, is been summarized in the form of fingerprint in Table No. 1. The table communicate presence or absence of phytochemical of different parts of *D. Roxburghii* in seven different solvents such as water, acetone, ethanol, methanol, chloroform, hexane and ether. The preliminary phytochemical screening of roots, stem and leaves of *D. roxburghii* has revealed that the different parts of plant are rich in medicinally active compounds like alkaloids, carbohydrates, glycosides, steroids, flavonoids, coumarins, saponins, fatty acids, tannins, protein and amino acids, gum and mucilage, terpenoids, anthroquinones and phenols. Fig: 1 depicts quantitative estimation (%) of secondary metabolites like Alkaloid, flavonoid, saponin, tannin, phenol and terpenoids in leaf, stem and roots of *Drypetes roxburghii*. To check the reproducibility, experiment was conducted 3 times and data is represented in the form of Mean± SD.

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Table 1- preliminary phytochemical fingerprint of *D. roxburghii*

Plant name	W	Ac	Et	Mt	Ch	Hx	Eth	Tests	Phytochemicals
Dr(leaf)								Dragendorff's	Alkaloids
Dr (stem)									
Dr (roots)									
Dr(leaf)								Mayer's test	
Dr (stem)									
Dr (roots)									
Dr(leaf)								Wagner's test	
Dr (stem)									
Dr (roots)									
DR(leaf)								Borntreger's test	Anthroquinones
DR (stem)									
DR (roots)									
Dr(leaf)								Molisch's test	Carbohydrates
Dr (stem)									
Dr (roots)									
Dr(leaf)								Barford's test	
Dr (stem)									
Dr (roots)									
Dr(leaf)								Benedicts test	
Dr (stem)									
Dr (roots)									
Dr(leaf)								Keller- Killiani test	Cardiac Glycosides
Dr (stem)									
Dr (roots)									
Dr(leaf)								Using NaOH	Coumarins
Dr (stem)									
Dr (roots)									
Dr(leaf)								Paper test	Fatty acids test
Dr (stem)									
Dr (roots)									
Dr(leaf)								Shinoda test	Flavonoids
Dr (stem)									
Dr (roots)									
Dr(leaf)								Alkaline reagent test	
Dr (stem)									
Dr (roots)									
Dr(leaf)								Lead acetate test	
Dr (stem)									
Dr (roots)									
Dr(leaf)									Gum and Mucilage
Dr (stem)									
Dr (roots)									
Dr(leaf)								Ninhydrin test	Protein and Amino
Dr (stem)									

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Dr (roots)									Acids
Dr(leaf)								Ferric chloride test	Phenols
Dr (stem)									
Dr (roots)									
Dr(leaf)								Frothing test	Saponins
Dr (stem)									
Dr (roots)									
Dr(leaf)								Liebermann Burchard reaction	Steroids
Dr (stem)									
Dr (roots)									
Dr(leaf)								Using FeCl3	Tannins test
Dr (stem)									
Dr (roots)									
Dr(leaf)								Salkowski test	Terpenoids
Dr (stem)									
Dr (roots)									

Dr= Drypetes roxburghii, W=water, Ac=Acetone, Et= Ethanol, Mt= Methanol, Ch= Chloroform, Hx=Hexane, Eth= ether, grey colour box = present, white colour box= absent

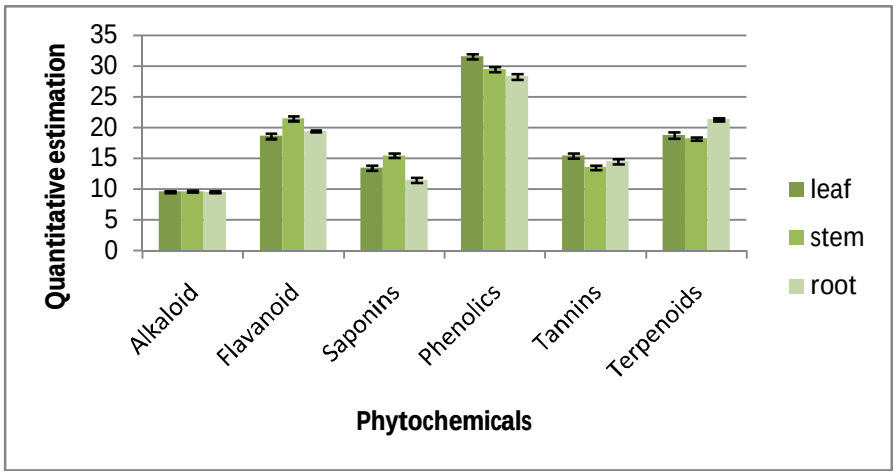

Fig: 1- Quantitative estimation (%) Secondary metabolites of different parts of *Drypetes roxburghii*, n=3, Data is presented as Mean± SD.

Separation of flavonoid fraction:

Silica gel chromatography was used to separate the flavonoid fractions. Total 60 fractions were eluted. Shinoda test confirmed the presence of flavonoids in the fractions 26-30, 35 - 40, 50-55. These fractions were pooled to form fraction I, II and III. And later these fractions were run on thin layer chromatography. Solvent system used was Ethyl acetate: Formic acid: water (8:0.1:0.5). In TLC, the number of bands, Rf value, colour of bands were recorded and has been mentioned in table: 2. The obtained data is depicted in fig.2.

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Table:-2 Rf values and colour development of TLC solvent systems of methanolic extract of *Drypetes roxburghii*

Sample code	TLC Band	Retention Factor	TLC Profile characteristics		
			Visible light (Figure 1)	Shortwave UV 254 nm (Figure 2)	Longwave UV 366 nm (Figure 3)
I	1	0.8	Brown	blue	Green
II	1	0.65	Light brown	Light blue	Light green
III	1	0.85	Yellowish brown	Light blue	Green

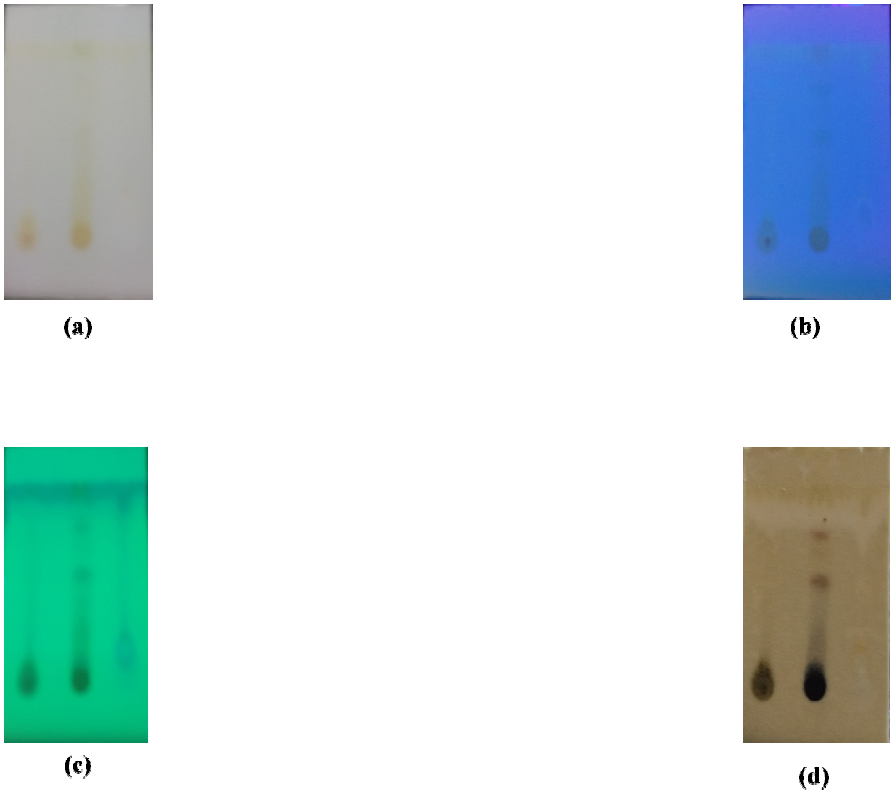

Fig 2: thin layer chromatography of methanolic leaf extract of *Drypetes roxburghii*, (a) TLC chromatogram (Visible Light), (b) TLC Chromatogram (254 nm) , (c) TLC Chromatogram (366 nm) and (d) 1% Ferric Chloride in 50% methanol (Spray)

Antibacterial activity of flavonoid fractions:

The antibacterial activity of isolated flavonoid fractions was detected by agar well diffusion method. The zone of inhibition determines the extent of anti-bacterial activity. All the isolated flavonoid fractions were found to have anti microbial activity but the degree of activity been found to be different. The activity was tested against pathogenic bacteria like *Escherichia Coli, Salmonella typhimurium* and *Pseudomonas aeroginosa and* fungi viz. *Apergillus fumigates, Apergillus niger* and *Microporum gypseum* The activity of the isolated fractions was compared with streptomycin and nystatin (standard antibiotics).

D. roxburghii was found to have good antimicrobial activity. The obtained results are portrayed in fig: 3. it compares the zone of inhibition of standard, fraction I, II, III and control against *E. Coli, S. Typhimurium, P. Aeroginosa, A. fumigates, A. niger* and *M. gypseum* In *D. roxburghii,* the standard antibiotic streptomycin showed the zone of inhibition of 21.33mm against *E.coli* and the three isolated fractions showed 11.33mm, 12mm and 11mm. The standard

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antibiotic streptomycin showed the zone of inhibition of 18mm against *S.typhimurium*. The isolated flavonoid fractions (I, II and III) of *D. roxburghii* showed 10.33, 11.33 and 10.66. The standard antibiotic streptomycin showed the zone of inhibition of 21.33mm against *P. aeroginosa*. The isolated flavonoid fractions (I, II and III) of *D. roxburghii* the three fractions showed 13mm, 9.66mm and 12mm.

For fungi, the standard antibiotic nystatin showed the zone of inhibition of 21.33mm against *A. fumigates*. The isolated flavonoid fractions (I, II and III) of *D. roxburghii* the three fractions showed 13.33mm, 14.66mm and 13.83mm. The standard antibiotic nystatin showed the zone of inhibition of 18mm against *A. niger*. The isolated flavonoid fractions (I, II and III) of *D. roxburghii* the three fractions showed 10.33 mm, 11.33 mm and 10.66 mm. The standard antibiotic nystatin showed the zone of inhibition of 21.33mm against *M.gypseum*. The isolated flavonoid fractions (I, II and III) of *D. roxburghii* the three fractions showed 13mm, 9.66mm and 12mm.

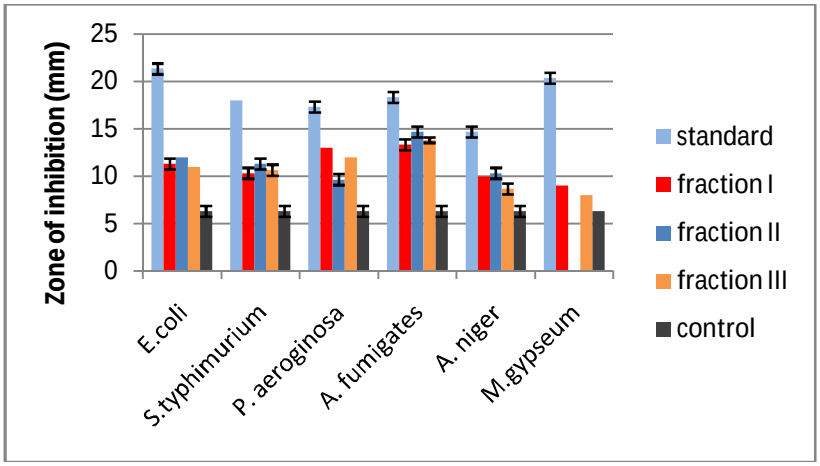

Fig 3: Antibacterial activity of Flavonoid fractions isolated from methanolic leaf extract of *Dryptes roxburghii*

IV. DISCUSSION

Phytochemicals are ubiquitous in plants and play multiple functions like protection, attraction of insects for seed dispersion and pollination. They are naturally occurring organic molecules. Environmental conditions can influence the level and types of phytochemicals present in plants. Due to the presence of phytochemicals, plant kingdom has proven to be the most useful in the treatment of diseases and they provide an important source of all the world's pharmaceuticals. The most important of these bioactive constituents of plants are steroids, terpenoids, carotenoids, flavanoids, alkaloids, tannins and glycosides. Plants in all facet of life have served a valuable starting material for drug development [27].

The number of higher plant species on this planet is estimated at 250,000 [28]. According to Schultes, 1978 [29], only 10% of total secondary metabolites have been isolated. Of these, only around 6% have been screened for biological activity and of which only 15% are reported to be phytochemically characterized [30]. Currently, plant based drugs are researched, dispensed, formulated and manufactured in modern framework rather than in the form of conventional dosage forms. Hence, it has becomes an important interface among various branches of pharmaceutical sciences. It is now emerging as interdisciplinary science that incorporates inputs from chemistry, biology and biotechnology directed towards natural products based drug discovery [31].

In an ethanobotanical research, different parts of *Drypetes roxburghii* is being used to treat many diseases, hence it is an excellent nominee for phytochemical analysis. The present study revealed the presence of medicinal active constituents of *D. roxburghii*. The phytochemical compounds of *D. roxburghii* are qualitatively analyzed for root, stem and leaf separately using seven different solvents and results are presented in Table 1. Qualitative study of alkaloids,

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carbohydrates, glycosides, steroids, flavonoids, coumarins, saponins, fatty acids, tannins, protein and amino Acids, gum and mucilage, terpenoids, anthroquinones and Phenols variation in the content in different organic solvents is depicted in Table 1.

Quantitative estimation of *Dryptes roxburghii* revealed that alkaloid is in between the range of (9.5 to 9.6%), flavonoids (18.63 to 21.23%), saponins (11.36 to 15.13%), phenolics (28.36 to 31.1%), tannins (13.16 to 15.38%) and terpenoids (18.8 to 21.56%). The estimated quantitative data is presented in Fig 1.

The standard antibiotic streptomycin showed the zone of inhibition of 21.33mm against *E. coli*. The isolated flavonoid fractions (I, II and III) of *Dryptes roxburghii* the three fractions showed 11.33mm, 12mm and 11mm. our findings does not match with The Audipudi & Chakicherla [32] findings. They reported the antimicrobial activity of flavonoid containing methanol extracts of *Gmelina arborea* against *S. typhi*, *S. aureus*, *Mycobacterium spp.* and *P. vulgaris* but found inactive against *E. coli*, whereas in the current investigation, the flavonoid fractions showed good antibacterial activity against *E. coli*. Similar work was carried by Vandana Mathur, [33] in *D. indica*, diethyl ether fraction of flavonoids of *D. indica* showed 2.5 mm inhibition zone (IZ) against *E. coli*. Ether fraction showed 4mm (IZ) and alcoholic fraction showed maximum 2 mm against *E. coli*.

The standard antibiotic streptomycin showed the zone of inhibition of 18 mm against *S. typhimurium*. The isolated flavonoid fractions (I, II and III) of *Dryptes roxburghii* the three fractions showed 10.33 mm, 11.33 mm and 10.66 mm. Our finding agrees with the findings of A. Doss et.al. [34], they carried antimicrobial work of flavonoid fractions obtained from *Mimosa pudica* and *Panicum* maximum against *S. typhimurium.* Flavonoid fraction of *Mimosa pudica* had shown zone of inhibition 10.0 mm and flavonoid fraction of *Panicum maximum* showed 17.1 mm zone of inhibition.

The standard antibiotic streptomycin showed the zone of inhibition of 21.33 mm against *P. aeroginosa*. The isolated flavonoid fractions (I, II and III) of *Dryptes roxburghii* the three fractions showed 13mm, 9.66mm and 12mm. Antibacterial activity of flavonoid against *P. aeruginosa* has also been mention by Hayet Edziri et al., [35] they had isolated two flavonoids, flavone and an isoflavone from the flowers of *R. raetam*, and recorded the zone of inhibition 20 mm against *P. aeruginosa.* And Elsa Varghese et al. [36] had also successfully isolated flavonoids from fruits of *Helicteres isora* Linn and recorded the zone of inhibition of 14mm against *Pseudomonas aeruginosa*.

The antifungal activity of isolated flavonoid fractions was detected by agar well diffusion method. The zone of inhibition determines the extent of anti-fungal activity. All the isolated flavonoid fractions were found to have anti microbial activity but the degree of activity been found to be different. The activity was tested against pathogen like *A. niger* and *A. fumigates* and *M.gypseum.*

The standard antibiotic nystatin showed the zone of inhibition of 21.33mm against *A. fumigates*. The isolated flavonoid fractions (I, II and III) of *Dryptes roxburghii* the three fractions showed 13.33mm, 14.66mm and 13.83mm. The standard antibiotic nystatin showed the zone of inhibition of 18mm against *A. niger*. The isolated flavonoid fractions (I, II and III) of *Dryptes roxburghii* the three fractions showed 10.33, 11.33.

V. CONCLUSION

The *Drypetes roxburghii* was screened for phytochemical constituents and antimicrobial activity. It was found to be good source of medicinally active constituents and turn out to be effective and potential source of advanced drugs. This work justifies the further need to isolate and characterize the medicinally active compounds.

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