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STUDY OF SECONDARY METABOLITE CONSTITUENTS OF SOME MEDICINALLY IMPORTANT PLANTS OF INDIAN WESTERN REGION FOREST

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Abstract: Medicinally important plants from Indian western region forest are a source of diverse bioactive compounds comprising to various groups including antimicrobial, anticancer, antispasmodics etc. A large number of the medicinally important plants are claimed to possess the antimicrobial and antibiotic properties in the traditional medicinal system and are also used by the local people for the treatment of various diseases. Extraction of the bio-active compounds from plants has always been a challenging task for the workers. Comparative studies of secondary metabolites using qualitative tests were performed on *Balanites aegyptiaca* Del. (Hingot), *Tecomella undulata* D.Don. (Rohida), *Ficus racemosa* Linn. (Gular) and *Capparis decidua* (Forssk.) Edgew (Kair). Extraction from powdered plants parts using different types of solvent like methanol, ethyl acetate, petroleum ether, acetic acid and water were done. The phytochemical screening including secondary metabolites revealed the presence of many chemical constituents including Alkaloids, Flavanoids, Steroids, Triterpenoids, Terpenoids, Tannins, Glycosides, Saponins, Coumarins, and Cardiac Glycosides etc. The result clearly indicates the richness of phytochemical in above mentioned plant species and thus could be potential source of bioactive compounds.

Index Terms **- antispasmodics, antibiotic, methanol, ethyl acetate, petroleum ether, acetic acid, Alkaloids, Flavanoids, Steroids, Triterpenoids, Terpenoids, Tannins, Glycosides, Saponins, Coumarins, and Cardiac Glycosides.**

I. INTRODUCTION

Bio-active compounds from plants have great importance and serve the very purpose among local people and now a day's these bio-active compounds has obtained greater attention of workers to prevent and treat the human diseases. Compounds from medicinally important plants are considered to be more bio-friendly. It is revealed that the over 6000 plants in India are used in traditional medicinal system and form the great part of herbal medicinal system and approx 75% medicinal needs of the third world countries are to be fulfilled by these traditionally important plants (Rajasekharan PE. 2002). Medicinally important plants from Indian western region frequently reported that they had therapeutic value for treating bacterial, fungal infection, fever, rheumatism, diarrhea, asthma and piles (Upadhyay B et al. 2007). The plant *Tecomella undulata* (Rohida) which is state plant of Rajasthan, locally used in treatment of Syphilis and leucorrhoea and the plant *Capparis decidua* (kair) used in treatment of Pyorrhoea, rheumatism and Muscular injuries etc by local people (Upadhyay B et al. 2007). The plant *Balanities aegyptiaca* (Hingot) used as Anthelmintic, alexipharmic, antidysentric, Analgesic and used in the treatment of Leucoderma, Ulcers, Skin diseases (Tripathi YC et al. 1996). The plant *Ficus racemosa* (Gular) is considered to be sacred grove and have great healing power and use in treatment of insect bites (Acharya CK 2015). These four medicinally important plants from Indian western region have been selected for the screening of bio-active compounds. Selected plant part on the basis of earlier knowledge has been picked out for the qualitative analysis of secondary metabolites. The method of extraction by using various solvent used pharmaceutically involves the separation of bio-active compounds from inactive or inert components of plant tissue. During the procedure of extraction, solvents diffuse into the solid plant material as their property to diffuse and solubilise the various compounds including secondary metabolites with similar polarity (Ncube NS et al. 2008). The plant extract obtained by using various solvents after purification can be used as medicinal agent such as topical solution and extract can be further processed to be form a active ingredients of tablets and capsules. These plant extracts contains complex mixture of many bio-active compounds including secondary metabolites such as alkaloids, glycosides, terpenoids, flavonoids etc. (Handa SS 2008). The bio-active compounds from plants serve as reducing agent for silver ions in the solution and the synthesis of nanoparticles might have resulted due to redox activities of these bio-active compounds. Therefore, the plants which are rich in secondary metabolites serve as suitable candidates for the synthesis of metallic nanoparticles (Jha AK and Prasad K 2010). However, the selected plants and their parts used for the extraction of bio-active compounds are less known to the literature of Indian Medicinal Plants (Upadhyay B et al. 2007, Tripathi YC et al. 1996, Acharya CK 2015, Ncube NS et al. 2008 and Handa SS 2008). The present study investigates the qualitative assessment of bio-active compounds for some medicinally important plants from Indian

Western region and screening of secondary metabolites such as Alkaloids, Flavanoids, Steroids, Triterpenoids, Terpenoids, Tannins, Glycosides, Saponins, Coumarins, and Cardiac Glycosides etc.

II. MATERIAL AND METHODS

2.1 Identification and collection of plant material

Plants from Indian Western region serve as effective phytomedicines since times immemorial; many workers revealed the extraction of various components from plants and use thereof in the production of medicinal compounds. The natural constituents or bioactive compounds from plants can be derived from any part of the plant like leaves, bark, flowers, fruits, mesocarp, roots, seeds etc. The selected medicinally important plants are collected from nearby areas. As the plant parts fruit mesocarp and leaf of the plant Balanites aegyptiaca were collected from Jhalan dungri forest, Jaipur, plant parts flower and leaf of the plant Tecomella undulata were collected from Department of Botany campus, University of Rajasthan, Jaipur, the plant parts whole fruit and bark of Ficus racemosa were collected from Nursery, University of Rajasthan, Jaipur and the plant parts bark and root of Capparis decidua were collected from Tordi hills, Malpura, Rajasthan. The collected material was authenticated and a voucher specimen was submitted in the Herbarium, Department of Botany, University of Rajasthan, Jaipur. Plant parts under study were washed; shade dried at room temperature for 3-5 days and finely powdered and was kept under ambient conditions for further use during study.

2.2 Selection of Solvent

The type solvent used for the extraction of bioactive compound plays a major role and successful determination of bio-active compounds from medicinally important plant is largely dependent on the type of solvent. A good solvent have many properties such as less toxicity, ease of evaporation, rapid absorption of the extract and inability to cause the extract to complex or dissociate (Eloff JN 1998). So the solvent such as Ethyl acetate, Methanol, Acetic acid, Petroleum ether and water extracts etc used for the extraction of bioactive compounds from medicinally important plants.

2.3 Preparation of plant extract

The dried and grinded powdered form of sample has been taken for the extraction of bio-active compounds. 50 ml of each solvent like petroleum ether, methanol, ethyl acetate and acetic acid were taken into sterilized conical flask of 100ml capacity. To get the supernatant of samples Whatman No.1 filter paper were used in which 5gm of each dried powder was used. The solvent to dry weight ratio of sample should be 10:1 (v/w), it is reported for the best results (Das K et al. 2010). These solvent and powder containing flasks were shaken at 200 rpm for 24 hr on rotary shaker at 37oC temperature (Central Facility, Department of Botany, University of Rajasthan, Jaipur). Later the extracts were filtered through Whatman No.1 filter paper. Supernatant were collected separately and dried in the Petri plates (pre-weighed) for 24-48 hrs for total crude extracts estimation. Estimation of crude extract by using different solvent id done by weighing the petri plates after 24-48 hrs (Table no. 1). These crude extract plant samples were labelled and stored for the further phytochemical analysis. Water (aqueous) extract was prepared fresh for biochemical analysis. 5 gm powdered plant sample was added to 50 ml of distilled water, shaking at 200 rpm for 30 min. at 37° C. then filtered with Whatman No.1 filter paper and used for further phytochemical analysis.

Table -1 showing the crude extract concentration using different solvents (conc. in mg/10gm plant sample.)

Plants	**B. aegyptiaca**		**C. decidua**		**F. racemosa**		**T. undulata**	
Solvents	**Fruit mesocarp**	**Leaf**	**Bark**	**root**	**Whole fruit**	**Bark**	**Flower**	**leaf**
Ethyl acetate	17.5	20.6	7.2	4.4	68.2	14.9	35.4	55.5
Pet. Ether	6.5	12.1	26	1.8	49.2	12.3	221.9	18.8
Acetic Acid	95.1	116.6	32.3	3.02	39.2	54.1	219.6	174.1
Methanol	177.5	125.4	13.3	3.04	48.0	15.4	229.2	279.6
Water	28.5	92.0	16.7	2.67	23.9	28.4	129.0	96.8

III. PHYTOCHEMICAL SCREENING

The phytochemical screening, for the detection of bioactive compounds was carried out with Ethyl acetate, Methanol, Acetic acid, Petroleum ether and water extracts. 100mg of each extract was dissolved in 50 ml of the respective solvent and filtered through Whatman No.1 filter paper. Thus, the filtrates obtained were used as test solutions for the following phytochemical screening tests (Edeoga HO et al. 2005, Tiwari P et al. 2011).

3.1 Test for Alkaloids (Mayer's test)

The residue of the extraction samples dissolved in the acidic solution (1.5 ml of HCl (2%), Mayer's reagent (Potassium mercuric iodide solution, prepared fresh) was added (2 drops). Cream coloured/White precipitate indicates the presence of alkaloids.

3.2 Test for Flavonoids (Ferric chloride test)

A few drops of neutral ferric chloride solution were added to 1 ml each of above plant extract solution. Formation of blackish red colour indicates the presence of flavonoids.

3.3 Test for Steroids (Salkowski's test)

About 100 mg of dried extract was dissolved in 2ml of chloroform. Addition of sulphuric acid to the sample by forming a lower layer. Detection of presence of steroidal ring observed through reddish brown colour at the interface.

3.4 Test for Triterpenoids

10 mg of extract was dissolved in 1 ml of chloroform. 1 ml of acetic anhydride was added. Following the addition of 2 ml conc. H2SO4. Formation of reddish violet colour to the lower level indicates the presence of triterpenoids.

3.5 Test for Terpenoids (Salkowski test)

Reaction for the detection of terpenoids is performed by addition of 2 ml of chloroform, and concentrated H2SO4 (3ml) to the 5ml of each plant extract and mixed slowly to form a layer. Formation of reddish-brown layer at the interface estimate the presence of terpenoids.

3.6 Test for Tannins (Ferric chloride test)

50 mg of extract was taken and a few drops of ferric chloride solution were added. A blackish precipitate indicates the presence of tannins.

3.7 Test for Glycosides (Keller Killiani's test)

About 100 mg of extract was dissolved in 1 ml of glacial acetic acid containing 1 drop of ferric chloride solution and then add underlayer with 1 ml of concentrated Sulphuric acid (H2SO4). Formation of two layers indicate the presence of glycoside, a lower reddish brown layer indicated the presence of a de-oxy sugar characteristic of cardenolides and upper bluish layer due to acetic acid.

3.8 Test for Saponins

50ml of an aqueous extract of samples taken and ass a drop of sodium bicarbonate. The mixture was shaken vigorously and kept for 3min. and a honey comb like froth was formed and observed, it shows the presence of saponins.

3.9 Test for Coumarins

1 ml of each various extracts was treated with alcoholic 10% sodium hydroxide (0.5 ml). Dark yellow colour shows the presence of coumarins.

3.10 Test for Cardiac glycosides (Keller-Killani test)

5ml of each plant part extracts was treated with 2 ml of glacial acetic acid containing one drop of ferric chloride solution and 1 ml of H2SO4 added. A brown ring of the interface indicates a de-oxysugar characteristic of cardenolides. A violet ring may appear below the brown ring, while in the acetic acid layer, a greenish ring may form just gradually throughout thin layer which shows the presence of Cardiac glycosides.

IV. Quantitative determination of Bioactive Constituents

4.1 Determination of Alkaloids

Powdered and weighed selected plant materials were taken in 100 ml Erlenmeyer flasks and added 50 ml distilled water for each 20g of the dried plant/tissue material (v/w) and 5 ml of 0.05 N sulphuric acid was added to it. The mixture was kept on a magnetic stirrer for 3-4 h and boiled gently for 25 min. Heavy magnesium oxide (2.5 g/g) was added to the mixture and again boiled gently for 20 min. It was cooled at room temperature. The mixture was filtered through Whatman filter paper 1 (having diameter-12.5 cm) (Kogan L et al. 1953). The filtrate was evaporated to dryness *in vacuo* and residue is the alkaloid, which was dried and weighed (Table 3)

4.2 Determination of Flavonoids

20 gm of each of the shade dried and powdered plant material (plant parts such as leaf, fruit mesocarp, and flowers) of selected plants were mixed with 80% methanol (100ml/g wt of test material) for 24 hrs. The methanol soluble fractions were filtered and dried *in vacuo*. Each of the aqueous concentrates was fractioned by sequential extraction with petroleum ether (Fraction-1), diethyl ether (Fraction-2), and ethyl acetate (Fraction-3) thrice (Subramanian SS and Nagarajan S 1969). Due to the presence of high fatty acids, Fraction 1 was discarded whereas Fraction-2 and fraction-3 were analyzed for free and bound flavonoids respectively. Fraction-2 was concentrated *in vacuo* and kept for quantitative analysis. Fraction 3 was hydrolyzed by refluxing with 7% sulphuric acid (10 ml/g residue) for 2 hrs, filtered, and filtrate extracted thrice with ethyl acetate. The ethyl acetate layer (bound flavonoid) of each extract was neutralized with distilled water and concentrated *in vacuo* and dried and weighed (Table 3)

4.3 Determination of phenols

The determination of total phenolic content in selected plant extracts (methanolic extract) was done by Folin-Ciocalteu's reagent method (Karim A et al. 2011). 0.5ml of each extract and 0.1 ml (0.5N) Folin-Ciocalteu's reagent was mixed thoroughly and leave at room temperature 280C for 15-20 min. Then 2.5 ml sodium carbonate solution was added to the mixture and again leave at room temperature 280C for 30 min. Then the absorbance was measured at 760 nm on a spectrophotometer. Gallic acid has been taken for positive control. The estimation of total phenol values is done in terms of Gallic acid equivalent (mg/gm of extracted constituents) (Table 3).

4.4 Determination of Tannin

The determination of tannin content in extract wad done by Van-Burden and Robinson method: 500 mg of the extract (methanolic) was weighed into a 50 ml flask. The addition of 50 ml of distilled water was done and shaken for approx 1 h in a shaker. The mixture was filtered and measured. Then 5 ml of the filtered was mixed with 2 ml of 0.1 M FeCl3 in 0.1 N HCl and 0.008 M potassium ferrocyanide to prepare the mixture for determination of phenolic content. The absorbance was measured at 120 nm on Spectro-photometer within 10 min (Van Buren JP and Robinson WB 1969). The crude extract is taken as reference (Table 3).

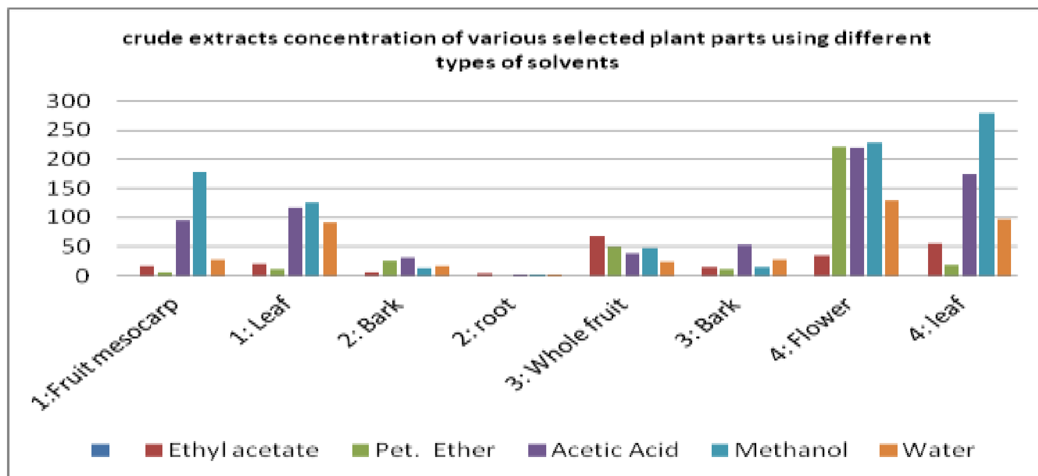
Table: 2 showing the qualitative estimation of selected plant's parts using different solvents.

Secondary metabolites	Plants→	B. aegyptiaca		C. deciduas		F. racemosa		T. undulata	
	Solvents	Mesocarp	Leaf	Bark	Root	Fruit	Bark	Flower	Leaf
1.Alkaloids	Ethyl acetate	+	+	-	+	-	+	+	+
	Pet. Ether	++	+++	+	+	-	++	++	+
	Acetic Acid	+	-	++	++	+	+	-	++
	Methanol	++	+++	+++	++	++	++	++	++
	Water	+	+	+	++	+	-	++	++
2.Flavanoids	Ethyl acetate	+++	++	++	++	+	+	+	++
	Pet. Ether	++	+	++	+	-	+	++	++
	Acetic Acid	+	-	++	++	+	+	+	+
	Methanol	++	+++	+++	++	+	++	++	++
	Water	+	++	++	-	-	+	+	++
3.Steroids	Ethyl acetate	++	+	++	++	+	-	+++	+++
	Pet. Ether	+++	++	+	+	-	+	++	++
	Acetic Acid	++	++	-	+	+	+	++	+
	Methanol	++	++	+++	+++	++	-	++	++
	Water	+	-	+	+	+	-	++	++
4.Triterpenoids	Ethyl acetate	++	++	-	-	++	+	+	+
	Pet. Ether	++	+	+	+	+	++	+	+
	Acetic Acid	+	++	+	+	+	+	+	-
	Methanol	++	++	++	+	+	+	++	+
	Water	++	++	++	+	+	+	+	-
5.Terpenoids	Ethyl acetate	+	+	+	+	+	+	++	++
	Pet. Ether	++	+	+	+	++	+	++	+
	Acetic Acid	+	-	+	++	+	+	+	+
	Methanol	+	++	+	++	+	-	+	+
	Water	+	+	+	-	+	++	++	++
6.Tannins	Ethyl acetate	+	+	+	-	+	-	++	++
	Pet. Ether	+	++	+	+	+	+	+	++
	Acetic Acid	+	+	++	+	-	-	++	++
	Methanol	+	++	++	-	+	+	+++	++
	Water	-	+	+	-	-	-	+	++
7.Glycosides	Ethyl acetate	++	++	+	+	+	++	+	+
	Pet. Ether	+++	++	+	++	++	+	++	++
	Acetic Acid	++	+	+	+	+	++	+	++
	Methanol	++	++	++	+++	+	++	++	++
	Water	++	++	+	+	+	+	+	+
8.Saponins	Ethyl acetate	+++	++	+	+	+	-	+	++
	Pet. Ether	+++	+	+	+	-	+	++	++
	Acetic Acid	++	++	-	++	-	-	++	+++
	Methanol	++	++	+	+	+	++	+++	++
	Water	+++	++	-	-	-	-	++	+
9.Coumarins	Ethyl acetate	-	+	+	+	-	+	++	+
	Pet. Ether	-	-	+	+	-	-	+	+
	Acetic Acid	-	+	++	+	-	-	+	+
	Methanol	+	+	++	++	-	-	+	+
	Water	-	-	+	+	-	-	+	++
10. Cardiac Glycosides	Ethyl acetate	++	++	+	+	+	++	+	+
	Pet. Ether	+++	++	+	++	++	+	+++	++
	Acetic Acid	++	+	++	+	++	++	++	++
	Methanol	++	++	++	++	+	++	++	++
	Water	+	+	+	+	+	++	++	++

Table 3. Percentage of crude alkaloids, phenols, tannin, flavonoids in the medicinally important plants from Indian western region.

Plants	B. aegyptiaca		C. deciduas		F. racemosa		T. undulata	
Bio-active compounds	Fruit mesocarp	Leaf	Bark	root	Whole fruit	Bark	Flower	leaf
Alkaloids %	0.70±0.10	0.85±0.12	0.64±0.16	0.35±0.10	0.29±0.12	0.39±0.10	0.69±0.15	0.94±0.11
Flavonoids %	0.86±0.20	0.91±0.14	0.92±0.13	0.85±0.10	0.35±0.14	0.40±0.11	0.77±0.12	0.75±0.15
Phenols %	0.39±0.14	0.32±0.16	0.28±0.11	0.18±0.10	0.08±0.10	0.06±0.11	0.21±0.13	0.34±0.20
Tannins %	0.15±0.12	0.10±0.12	0.19±0.12	0.20±0.16	0.78±0.11	0.34±0.11	0.21±0.15	0.19±0.10

Graph 1: Showing the crude extracts concentration of various selected plant parts using different types of solvents (1: *B. aegyptiaca*, 2: *C. decidua,* 3: *F. racemosa*, 4: *T. undulata*)



V. RESULTS AND DISCUSSION

The present study carried out on the four plant species selected from Indian western region revealed the presence of many bioactive compounds. The qualitative estimation of phyto-chemicals of four medicinally important plants investigated is summarized in Tables 2. In the present study a comparative analysis was made to determine secondary metabolites alkaloids, flavanoids, phenolics, sterols, triterpenoids, tannins, terpenoids, glycosides and cardiac glycosides qualitatively. External stimuli such as microbial infections, stress conditions like drought, salt, high temperature, ultraviolet radiation, and chemical stressors induce their synthesis. Many ecologic functions include defence against microbial pathogens and herbivorous animals also stimulate the plants to secrete these bio-active compounds. The plant secondary metabolites or bioactive compounds such as Alkaloids, Flavanoids, Steroids, Triterpenoids, Terpenoids, Tannins, Glycosides, Saponins and Cardiac Glycosides are present in almost all four plant selected for study, the Ficus racemosa samples negative for the coumarins, and rest are positive for coumarins (Table 2). The qualitative analysis of bioactive compounds and intensity of amount detected in samples shows that the plant sample of Balanites aegyptiaca and Tecomella undulata were found to be higher among the four plant species selected for study (Graph 1) and Solvent Methanol and Acetic acid were found to be best solvent for extraction of secondary metabolites and bioactive compounds. The Quantitative analysis of secondary metabolites such as alkaloids, flavanoids, phenolics and tannins was done and summarized in Table 3. The estimation of secondary metabolites assay was carried out in triplicate and the mean values with ± SEM are presented. The extract of leaves of Tecomella undulata and Balanites aegyptiaca contained the highest percentage crude yield of alkaloids (0.94%) and (0.85%) respectively, while extract of fruit Ficus racemosa contained the lowest yield of alkaloid (0.29%) but the highest yield of tannin (0.78%). Phenols were obtained in the plants but the yields recorded were maximum in Fruit mesocarp extract of Balanites aegyptiaca (0.39%). Bark extract of Capparis decidua content the highest flavonoid content among four (0.92%). The present study showed that the leaves of plants rich in alkaloids and flavonoids, fruit of Ficus racemosa rich in tannin content. It is already known that methanolic extract of plant part of Tecomella undulata have analgesic and anti-inflammatory activities (Ahmad F et al. 1994) and have antimicrobial activities (Parekh J et al. 2006). Earlier it also reported that fruit extract of Balanites aegyptiaca have antimicrobial properties against wide range of bacteria (Al Ashaal HA et al. 2010) and leaf extract of same plant is also having antimicrobial properties (Doughari JH et al. 2007). Capparis decidua is also reported to exhibiting antimicrobial as well as antifungal activities (Keymanesh K et al. 2009). Further the plants rich in secondary metabolites promises to produce the efficient metallic nanoparticle having antimicrobial properties (Raut RW et al. 2010). The functional group of these secondary metabolites serve as binding agent for the metal like Ag, Au etc to produce the metallic nanoparticles, it means more the functional group, more the metal will be reduce to form nanoparticles (Raut RW et al. 2010). The plants selected for the qualitative and quantitative analysis here can be seen as a potential source of useful drugs. Further studies are going on these medicinally important plants in order to isolate and characterize the structure of the bioactive compounds. The antibacterial and antifungal activities of these selected plants for the treatments of the several diseases as claimed under traditional medicinal system are also being investigated.

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