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PHARMACOGNOSY COMPARISON BETWEEN *Saraca asoca* (Roxb.) AND *Polyalthia longifolia* (sonn.) from RATNAGIRI (M.S.)

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ABSTRACT

*Saraca asoca (Roxb.)*is a most ancient tree in India, It is used as a traditional medicines. It frequently known as Ashoka.it is belongs to family Caselpinaceae. In present study work with done in comparison between *Saraca asoca* (Roxb.) and *Polyalthia longifolia* (sonn.) with respect to pharmacognosy. Present study shows that,the glycoside present in both the selected species. The presence investigation shows various concentration of alkaloid, flavonoids and saponin like 1.56, 0.55, 3.56 gm/100gm respectively in *Saraca asoca (Roxb.)* 0.71, 0.37, and 4.30gm in *Polyalthia longifolia (Sonn.)* bark. The present investigation revealed that the various phytochemical components such as carbohydrates, flavonoids, saponin, tannin, phenol, glycoside and sterols are present in *Saraca asoca (Roxb.).*It has antimicrobial activity against various microorganism such as *Bacillus subtilis, Klebsiella pneumoniae, Staphylococcus aureus, and Pseudomonas aeroginosa and E.coli*.

Keywords- Phytochemistry, *Saraca asoca (Roxb.), Polyalthia longifolia* (sonn.)

INTRODUCTION-

Pharmacognosy is the knowledge of drug or pharmaceutical which deals with the drug of vegetables, animals and mineral origin. Pharmacognosy help to study the identification of source of materials forming drug description of its morphology and anatomy investigation of its potency, purity and freedom from admixture. In anatomy fundamental aspects to understanding of many aspects of biology. The

selected plant species are to find relationship between them.

Saraca asoca (Roxb.) has been greater used as traditional medicines. It is a most ancient tree in India, frequently known as Ashoka.it is belongs to family Caselpinaceae. In present study work with done in comparison between *Saraca asoca* (Roxb.) and *Polyalthia longifolia (sonn.)* with respect to pharmacognosy.

MATERIAL AND METHODS-

Material-

We have selected two plants such as *Saraca asoca* (Roxb.) and *Polyalthia longifolia (sonn.)*

Method-

i.**Collection of plant materials**- The collection of bark of (Roxb.) and *Polyalthia longifolia (sonn.)* from Jalgaon on 25 and 26 Oct. 2018

ii. **Exatraction of plant materials**-

Plant samples were washed with water and air-dried at room temperature for seven days. Oven dried at 40^0C to remove the residual moisture. The dried bark was powdered using a mixture grinder and stored in airtight container for future use. Five different solvents such as Acetone, diethyl ether, distilled water, Methanol and petroleum ether were used for extraction. About 1 gm of the plant samples were added respectively in to the test tubes containing with 5 ml solvents. Extracts were stored at room temperature.

Qualitative phytochemical analysis-

The extracts of all the five solvents of barks were tested for the presence of biological compounds by using following standard methods by (Sofowra, A. 1995. Trease G.E. et. Ali. 1989, Harborne J.B. 1973

a)Test for Carbohydrates-

i)Fehlings test –

Equal volume of Fehling A and Fehling B reagents were mixed together and 2 ml of it was added to crude extract and gently boiled. A brick red precipitate appeared at the bottom of the test tube indicated the presence of reducing sugars.

ii) Benedicts test-

Crude extract when mixed with 2 ml of Benedicts reagent and boiled, a reddish brown precipitate formed which indicated the presence of the carbohydrates.

iii) Iodine test-

Crude extract was mixed with 2ml of iodine solution. A dark blue or purple coloration indicated the presence of the carbohydrates.

b.)Test for phenols and Tannins-

Crude extracts were mixed with 2ml of 2% solution of $FeCl_3$. A blue-green or black coloration indicated the presence of phenol and tannin.

c.)Test for Flavonoids –

Alkaline reagent test- Crude extract were mixed with 2ml of 2% solution of NaOH. An intense yellow color was formed which turned colorless. An addition of few drops of diluted acid which indicated the presence of flavonoids.

d.)Test of saponins-

Crude extracts were mixed with 5ml of distilled water in a test tube and it was shaken vigorously. The formation of stable foam was taken as an indication for the presence of saponins.

e) Test for Glycosides-

Crude extracts were mixed with each of 2ml of chloroform and 2ml of acetic acid. The mixture was cooled in ice. Carefully concentrated H_2SO_4 was added. A color change from violet to blue to green indicated the presence of steroidal nucleus i.e.glycone portion of glycoside.

i.)Salkowskis test-

Crude extracts were mixed with 2ml of chloroform. Then 2 ml of concentrated H_2SO_4 was added carefully and shake gently.A reddish brown color indicated the presence of steroidal ring.i.e. glycogen portion of the glycoside.

ii)Keller-kilani test-

Crude extracts were mixed with 2ml of glacial acetic acid containing 1-2 drops of 2% solution of $FeCl_3$.The mixture was then poured in to another test tube containing 2ml of concentrated H_2SO_4. A brown ring at the interphone indicated the presence of cardiac glycoside.

f) Test for steroid-

Crude extract were mixed with 2ml of chloroform and concentrated H_2SO_4 was added sidewise. A red color produced in the lower chloroform layer indicates the presence of steroid.

g) Test for phenol compound-

The extracts were dissolved in 5ml of distilled water. To this, few drops of neutral 5% ferric chloride solution were added. A dark green color indicated the presence of phenol compounds.

Quantitative determination of phytochemicals-

a. Alkaloid determination using Harborne (1973) method-

2.5gm of the sample was added with 100ml of 5% acetic acid in ethanol and allowed to stand for 4 hour. The filtered extract was concentrated on a water bath to quarter of the original volume. Concentrated ammonium hydroxide was added until the precipitation was complete and allowed to settle. The precipitate was collected and washed with dilute ammonium hydroxide and the filtered. The residue is the alkaloid, which was dried and weighed.

b.)Flavonoid determination by the method of Bohm and Kocipai-Abyazan (1974)

2.5gm of each plant sample was weighed and 50ml of the 40% aqueous methanol was added at room temperature and shaken for 4 hours. The entire solutions was filtered through what man filter paper no.42 and repeat the process. The filtrate as a whole was transferred in to a crucible and evaporated to dryness over a water bath and weighed.

c.)Saponin determination-

For determination of saponin, 5g of each plant sample was weighed and was dispersed in 100ml of 20% ethanol. The suspension was heated over a hot water bath for 4 hour with continuous stirring at about 55^{0C}. The filtrate and residue were re-extracted with 100ml of 20% ethanol. The combined extracts were reduced to 40ml using water bath at temperature about 90^{0C} . The concentration was transferred in to a 250ml separating funnel and 20ml of diethyl ether was added and shaken vigorously. The aqueous layer was recovered and the purification process was repeated, about 30 ml on n-butanol was added. The combined n-butanol extracts were washed twice with 10ml of 5% aqueous sodium chloride. The remaining solution was heated in a water bath. After evaporation, the sample was dried in the oven to constant weight. The saponin content was calculated.

Thin Layer Chromatography-(Poonam S. Mohad (2014)

Thin layer chromatography of the extracts was done in TLC plates-2-5ug of 1% solution of sample spotted using micro pipette. Various solvents like acetone, butanol and acetone-butanol (1:1).Plate is placed under UV light, dark spots are observed.

The Rf value of sample was calculated by using formula, The five sequential extracts are used for TLC profiling. Before spotting the extracts are filtered and concentrated, in order to remove the solvents.

Determination of Antibacterial Activity-

a. Collection of Test Organisms-

The test organisms were collected from nearby diagnostic laboratories including *Bacillus subtilis, Klebsiella pneumoniae, Staphylococcus aureus, and Pseudomonas aeroginosa and E.coli.*

Antibacterial Activity test-

Test organisms were uniformly inoculated into Muller Hinton infusion agar plates. Filter paper discs were soaked in crude extracts of bark, it was placed on plates and zone of inhibition was measured in millimeter after 24 hrs incubation at 37^{0C}.

RESULT AND DISCUSSION-

Table No.01: Qualitative phytochemical analysis of *Saraca asoca (Roxb.)* bark and *Polyalthia longifolia (Sonn.)* bark (Presence of constituent= + , Absence of constituent= -)

	Saraca asoca (Roxb.) bark					*Polyalthia longifolia (Sonn.)* bark				
Test	**Acetone**	**Diethyl ether**	**D.W.**	**Methanol**	**Petroleum ether**	**Acetone**	**Diethyl ether**	**D.W.**	**Methanol**	**Petroleum ether**
Test for Carbohydrates										
Benedicts test	-	-	-	-	-	-	-	-	-	-
Fehlings test	-	-	-	-	-	-	-	-	-	
Iodine test	-	-	-	-	+	-	-	-	-	+
Test for flavonoids										
Alkaline reagent test	+	-	-	+	-	+	-	-	+	-
Test for saponins										
Froth foam test	-	-	-	-	+	-	-	-	-	+
Test for phenol and tannins										
Ferric chloride test	+	-	-	+	-	+	-	-	+	-
Test for Glycoside										
Liebermanns test	+	-	-	+	-	+	-	-	+	-
Salkowskis test	+	+	+	-	-	+	+	+	-	-
Keller-kilani test	+	-	-	+	-	+	-	-	+	-
Test for steroids										
Salkowskis test	+	-	-	+	-	-	-	+	+	-
Test for phenolic compounds										
Ferric chloride test	-	-	+	-	-	-	-	+	-	-

Phytochemical analysis conducted on selected plants. The extracts revealed the presence of constituents which are known to exhibit medicinal as well as physiological activities. The presence of phytochemical such as phenol, tannins, flavonoids, saponin, glycoside, phenolic compound contains alkaloid. The carbohydrates was found in all extracts of *Saraca asoca (Roxb.)* bark but *Polyalthia longifolia (Sonn.)* bark. Our results are in parallel to Bhadauria and *et.al*. (2012). There was absence of carbohydrate were shown in acetone, D.W. Methanol were shown in *Saraca asoca (Roxb.)* bark but *Polyalthia longifolia (Sonn.)* The glycoside present in both the selected species. The presence investigation shows various concentration of alkaloid, flavonoids and saponin like 1.56, 0.55, 3.56 gm/100gm respectively in *Saraca asoca (Roxb.)* 0.71, 0.37, and 4.30gm in *Polyalthia longifolia (Sonn.)* respectively as shown in table no.02

Table No. 02: Quantitative phytochemical analysis of *Saraca asoca (Roxb.)*) and*Polyalthia longifolia (Sonn.)*

Analysis	***Saraca asoca (Roxb.)* bark (gm/100gm)**	***Polyalthia longifolia (Sonn.)* bark (gm/100gm)**
Alkaloid	**1.56**	**0.91**
Flavonoid	**0.55**	**0.37**
Saponin	**3.56**	**4.3**

Table No. -03: Antimicrobial activity of *Saraca asoca (Roxb.)* bark and *Polyalthia longifolia (Sonn.)* bark

Organism	*Saraca asoca (Roxb.)* bark (Extract in solvent) Zone of inhibition					*Polyalthia longifolia (Sonn.)* bark (Extract in solvent) Zone of inhibition				
	Acetone	Diethyl ether	D.W.	Methanol	Petroleum ether	Acetone	Diethyl ether	D.W.	Methanol	Petroleum ether
Klebsiella pneumoniae	09	00	00	08	10	09	06	00	10	00
Pseudomonas aeroginosa	00	00	00	11	00	00	09	00	00	00
Staphylococcus aureus	00	00	00	00	00	00	00	27	00	00
Bacillus subtilis	08	00	00	00	00	09	00	22	00	00
E.coli	10	00	00	20	00	00	00	00	00	00

The antimicrobial activity of acetone extract of bark was highest on *E.coli* with zone of inhibition of 11mm and *Klebsiella pneumoniae* with zone of inhibition of 9 mm, while the lowest activity was noticed with acetone extract against *Pseudomonas aeroginosa* and *Staphylococcus aureus* and methanol extract of bark with zone of inhibition of 11mm.

The diethyl ether extract was least activity against all bacterial species. Petroleum ether extract showed no other activity on one species and also distilled water extracts has no antibacterial activity of *Saraca asoca (Roxb.)*.

The antimicrobial activity of bark extract, in acetone extract was highest on *Bacillus subtilis* with zone of inhibition is 9mm and no antimicrobial activity against other microorganism. Diethyl ether extract showed highest activity against *Pseudomonas aeroginosa*, the zone of inhibition was 27mm and activity showed against other microorganisms and distilled water extract highest activity against *Bacillus subtilis*, highest zone was showed against with the zone of inhibition of 22mm and lowest activity showed in *Polyalthia longifolia (Sonn.)*

The bioactive screening and antimicrobial activity of bark from selected plant species on chosen microbes concluded that presence of carbohydrate, tannin, alkaloid, saponin, flavonoids, glycoside, steroid, terpenoid in *Saraca asoca (Roxb.)* has an antimicrobial activity against *Klebsiella pneumoniae*., *Bacillus subtilis* and inactive result against *E.coli*. Our result is an parallel to Singh and *et al* (2019)

Table No.04: Thin layer chromatography of *Saraca asoca (Roxb.)* bark and *Polyalthia longifolia (Sonn.)* bark

Solvent	*Saraca asoca (Roxb.)*bark (Extract in solvent)					*Polyalthia longifolia (Sonn.)* bark(Extract in solvent)				
	Acetone	Diethyl ether	D.W.	Methanol	Petroleum ether	Acetone	Diethyl ether	D.W.	Methanol	Petroleum ether
Acetone	0.83	0.91	0.58	0.83	0.75	0.70	0.77	0.60	0.83	0.76
Butanol	0.74	0.88	0.75	0.85	o.66	0.75	0.67	0.88	0.81	0.80
Acetone:Butanol	0.89	0.87	0.86	0.74	0.86	0.72	0.69	0.83	0.87	0.70

The T.L.C. profile of both the selected species was carried out by using extracts of Acetone, Diethyl ether, D.W., Ethanol, Petroleum ether respectively. The different Rf values are observed in each solvents. Several spots on chromatography plate indicating presence of different fatty acids.

CONCLUSION-

Saraca asoca (Roxb.) is highly regarded as an universal panacea in ayurvedic medicine. It is one of the universal plant having medicinal activities and source of various type of compound. The present investigation revealed that the various phytochemical components such as carbohydrates, flavonoids, saponin, tannin , phenol, glycoside and sterols are present in *Saraca asoca (Roxb.).*It has antimicrobial activity against various microorganism such as *Bacillus subtilis, Klebsiella pneumoniae, Staphylococcus aureus, and Pseudomonas aeroginosa and E.coli.* It has used in many nontoxic traditional plant. The of use of phytoconstituents of asoca plant against diseases in challenge of development of modern drug discovery.

Polyalthia longifolia (Sonn.) has also some medicinal uses.it has the medicinal activities and source of various type of compound. The phytochemical component like phenol, glycoside etc. it has the antimicrobial activity against microorganism like *Bacillus subtilis, Klebsiella pneumoniae, Staphylococcus aureus, and Pseudomonas aeroginosa.*

Because of the destructive nature of plants, organization of cultivation programmes. The availability of crude drug is diminishing and this has reduced in the sale of adulterant. The commonly used adulterant in bark of *Polyalthia longifolia (Sonn.)* in Marathi called as khotaashok. The bark is mixed and that is reported by S.V. Lal in 1953 that both plants *Polyalthia longifolia (Sonn.) and Saraca asoca (Roxb.).*Contain two pharmacologically active fraction which have similar action on pain muscle through the mode of action of stimulant fraction is different in each case thus he concluded that stimulant fraction of *Saraca asoca (Roxb.* act by liberation of acetyl chloride that *Polyalthia longifolia (Sonn.)* Act directly on pain muscle fiber. As the same methods of the values are reported by Borkar A. (2017)

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