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A phytochemical and physicochemical investigation of *Bixa orellana* L. collected from Ranchi District, Jharkhand

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Abstract- *Bixa orellana* L. is commonly known as lipstick tree or sinduri belongs to the family Bixaceae. It is good medicinal properties and also used in industry as dye. The present study focused to analyzed extractive value, ash value and phytochemicals of leaf, bark and stem of *Bixa orellana* L. Extractive value was higher in methanolic extract of bark (12.67±0.41) as compare to the other plant parts. In total ash value and acid insoluble ash value was highest in leaf. The phytochemical screening of methanolic and aqueous plant extract was conducted to detect the presences of ten bioactive compounds including alkaloids, phenols, flavonoids, carbohydrate, protein, starch, terpenoids, tannins, saponins and glycosides. The determination of ash and extractive values provides information about the purity, potency, and consistency of plant drugs, will be helpful for standardization of drug.

Key words: Ash Value, Extractive Value, Phytochemical, Leaf, Stem, Bark

INTRODUCTION

Bixa orellana L. belonging to the family Bixaceae, known as Sinduri, Lipstick tree, achiote, annatto is small evergreen tree of central and Southern America.[1] It is found in mainly in the Brazilian forest ecosystems with humid tropical condition and now cultivated all over the world.[2] In India, it is commonly known as "sindoor".

Bixa orellana L. is a shrub or bushy tree which ranges from 3 to 10 meters in height.[3] Leaves are green colored, white or pink flower and red-orange seed in colour enclosed within capsule.[4] It has economic significance since it is a source of natural reddish orange pigment produced from the aril portion of the seeds. In global market, it is the most preferred natural food colorant. It is widely used as a colorant in butter, cheese, dairy products and chocolates.[5] It is also used as dyeing of wool, cotton, silk. Traditionally The Amazon tribes of Brazil generally use seed extracts to paint their body which also claims to repel insects as evidenced through studies in Wajapi tribal women of Brazil.[1,6]

Medicinal plant constitutes the main source of new pharmaceuticals and healthcare products.[7] *Bixa orellana* plant parts used as lowered the blood glucose levels in fasting normoglycemic and streptozotocin-induced diabetic dogs.[8] The root is used as controlling asthma, venereal diseases, dysentery, influenza and jaundice. The root bark is also used in gonorrhea, antipyretic, antiperiodic and antispasmolytic activities.[9] The extractive values in different solvents indicate the amount and nature of constituents in the extracts.[10] Previous phytochemical investigations have revealed the presence of several carotenoid compounds including bixin and norbixin some terpenoids, tocotrienols, and flavonoids in *Bixa orellana* seeds.[11] Secondary metabolites of plants play important role in health and

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nutritional important.[12] Screening of phytochemical revealed the presences of alkaloids, tannins, flavonoids, glycosides, saponins, terpenoids etc. Many plants extracts and phytochemicals show antioxidant properties.[13,14] Secondary metabolites of plants serve as defense mechanism against microorganisms and insects.[15]

The present investigation deals with the extractive value, ash value and preliminary phytochemical analysis in different solvent of leaf, bark and stem of *B. orellana* L.

METHODS

Plant collection

The fresh and healthy leaves, barks and stem of *Bixa orellana* were collected from Birsa Agriculture University, Kanke, Ranchi, Jharkhand. Plant materials were washed in running tap water and shade dried for 3-4 days and then kept under hot air oven at 37°C for 6 hours. The samples were powdered using motor pastel or mixer grinder and stored in a closed air tight container for further use.

Extractive Value

One gram of dry powered plant material was soaked in 20ml of different solvent for 24 hours. The extract was filtered through Whatman no.1 filter paper. The filtrates collected in separate pre-weighted evaporating dishes were evaporated to dryness on a room temperature. The residues obtained and weighed. The percentage of extractive value (w/w) was determined using the formula.[16]

$$\%\text{Extractive value} = \frac{\text{Wt. of the residue}}{\text{1g (dry powder plant material}} \times 100$$

Ash Values

Percentage of total ash value, acid soluble ash value and acid-insoluble ash values of the powdered of leaf, bark stem were performed as per Kokate standard procedure and result showed in table 2.

Preparation for extracts and phytochemical screening

Extracts of plant material were performed by cold extraction method. 5 gm powder of leaf, bark and stem separately dissolved in 50ml aqueous and methanol then left for 24 hours and filtrate it. Preliminary phytochemical screening of plant extract was carried out by the standard methods to observed active constituents.[16-18]

- **Test for Alkaloids**- 5ml of leaves extract and add 2-3 drop of Dagendroff's reagent. Presences of alkaloid were confirmed by the formation of reddish-brown precipitate.
- **Test for Carbohydrate**- 5ml of leaves extract and add 2-3 drop of Molish reagent along with this add 1ml of concentrate sulphuric acid (H_2So_4) down the slide of the test tube. Then allow the mixture to stand for 2-3min. Observe for the formation of red or dull violet colour at the interface of the two layers of positive result.
- **Test for Protein-** 5ml of extract and add 2 drops of millon's reagent. Presences of protein were confirmed by the formation of white precipitate.
- **Test for Flavonoids**- Take 5ml of extract and treat it with lead acetate solution. Observe for the formation of reddish-brown colour precipitate indicates the presence of flavonoids.
- **Test for Phenol**- 5ml of extract and treat it with 3-4 drop of 5% of aqueous ferric chloride solution. Presence of phenol was confirmed by the indication of blue-black colour.
- **Test for Terpenoids**- 5ml of extract and treat it with 2ml of chloroform and then add 3ml of concentrated sulphuric acid (H_2SO_4). Presence of Terpenoids was confirmed by the indication of reddish-brown colour.
- **Test of Glycosides**- 5ml of extract and treat it with glacial acetic acid and add few drops of 5% of $Fecl_3$. To this mixture add 0.5ml of concentrated sulphuric acid (H_2So_4). Two layer formation indicates glycosides, upper layer appear bluish green colour and violet ring appear at bottom.
- **Test for Saponin**- 5ml of extract and add distilled water and shake vigorously. Observe for the formation of persistence foam for 10-15 min that confirm the presence of saponin.
- **Test for Tannins-** 5ml of extract and added 10ml bromine water. Observe decolouration of bromine water confirm presence of tannin.
- **Test for Starch**– 5ml of extract and added few drops of dilute iodine solution. Presences of starch was confirmed by the indication of blue or black colour.

RESULT

Extractive value

The extractive value of *Bixa orellana* Leaf, bark and stem in methanol and aqueous. The comparison between them showed significant extractive value in methanolic extract. Extractive value was determined in triplicates and summarized in table 1.

Ash Value

Total ash value and acid insoluble ash value were highest in leaf. Acid soluble ash value was highest in stem showed in table 2.

Qualitative screening of phytochemicals

The phytochemical analysis gave an overview of which type of secondary metabolites are present in the extracts. Preliminary phytochemical screening was conducted ten different chemical compounds. Aqueous and methanol extract obtained from leaf, bark and stem. Extract from leaves showed some negative result which indicated that some phytochemicals were not detected in particular extract. In aqueous extract of stem showed all the phytochemicals. The result preliminary phytochemical screening showed in Table 3.

Table 1: Percentage of extractive value of different plant parts

Plant parts	Solvent	
	Methanol	Aqueous
Leaf	6.67±0.33	4.33±1.67
Bark	**12.67±0.41**	4.65±0.88
Stem	3.67±0.82	**4.67±0.67**

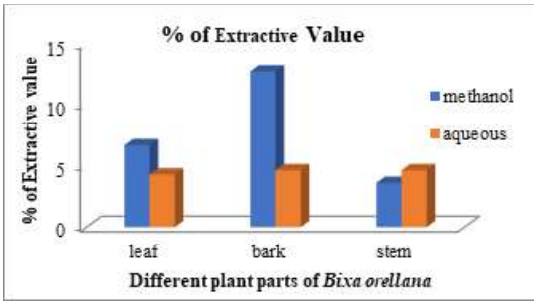

Fig 1: Graph showing percentage of extractive value

Table 2: Ash value of different parts of *Bixa orellana*

Sl. No.	Plant Parts	Total Ash Value	Acid Insoluble Ash Value	Acid Soluble Ash Value
1	Leaf	**6.33±0.33**	**5.00±0.00**	1.33±0.33
2	Bark	5.67±0.67	4.33±0.33	1.33±0.33
3	Stem	5.00±0.58	2.67±0.33	**2.33±0.88**

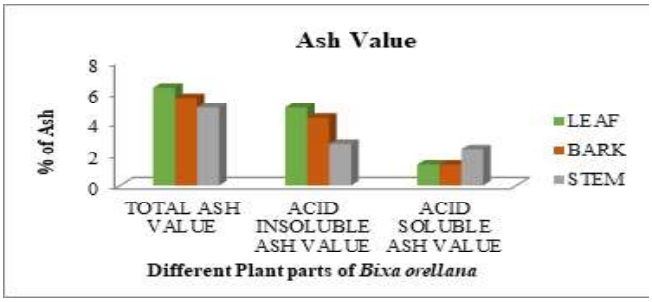

Fig. 2- Graph of Ash value of different parts of *Bixa orellana*

Table 3- Preliminary phytochemical screening of leaf, bark and stem

Sl. No.	Phytochemicals	Test	Leaf		Bark		Stem	
			M	Aq	M	Aq	M	Aq
1.	Alkaloids	Dagendroff's reagent	+	-	+	+	+	+
2.	Phenols	Ferric chloride test	+	+	+	+	+	+
3.	Flavonoids	Lead acetate test	+	-	-	-	+	+
4.	Starch	Iodine test	-	+	-	+	-	+
5.	Glycosides	Keller-killani test	+	+	-	+	-	+
6.	Tannins	Bromine water test	+	+	-	+	+	+
7.	Terpenoids	Salkowski's test	-	+	+	+	+	+
8.	Saponins	Foam formation	+	+	-	+	+	+
9.	Carbohydrate	Molish's test	-	+	+	+	+	+
10.	Protein	Millon's test	+	+	+	+	+	+

Note:- + Presence, - Absences, M- Methanol extract, Aq- Aqueous extract

CONCLUSION

The extractive value showed highest result in methanolic extract as compare to aqueous extract (table 1), it suggests that methanol is more effective at extracting certain bioactive compounds and thus can be used as preferred solvent for preparing extracts. Ash value helpful in determination of the quality and purity of crude drugs and also helpful in differentiation between genuine and substituted drugs.

This study contributes valuable data on the physicochemical and phytochemical screening of *Bixa orellana*, it supporting the need for future research into adulteration of drug and its bioactive compounds for potential therapeutic uses.

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