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Preliminary screening and report of the phytochemical and secondary metabolites present in *Byttneria aspera* colebr. Ex. wall

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Abstract

The leaves and stem of *Byttneria aspera* were dried in shade and subjected to methanolic extraction using soxhlet apparatus. The extracted plant samples were tested for the phytochemical contents as well as secondary metabolites.

Keywords: *Byttneria aspera*, shade dried-leaves/ stem, methanol extract, phytochemical testing

Introduction

The people in Mizoram practice traditional herbal medicines, the local knowledge of which has been descending through generations since time immemorial. Ethno medicinal plants are still widely used for curing different diseases both in urban and rural areas. There is a need for documentation of such valuable indigenous knowledge and domestication of economically important medicinal plants to decrease pressure over natural resources and to fulfil the requirements of national and local needs. (Lalramnghinglova, H., 2003)

In India, due to the tropical climate and poor living conditions of a large percentage of the population, the diseases caused by Fungi forms a major part in infection. (Sharma, SC., Syed, A.A., 2000)

There have been quite a number of medicinal plants identified to have certain actions toward different types of fungal species causing dermatophytoses, cutaneous infections, sub-cutaneous infections as well as superficial infections. Out of these many infections, the tribes of Mizoram have believed from ancient times that certain plants like *Terminalia belecayroxb* (Chawngkunga, C., 1996) [4], *Byttneria aspera* (Rozika, R., 2006) [3], *Tabernaemontana divaricate* (Rozika, R., 2006) [3] has the ability to fight against *candida sps* which causes *Candidiasis* apart from the many other Medicinal plants known to have effects on *Candida*.

Byttneria aspera has been used since time immemorial, as a remedy for oral thrush and mouth ulcers by the people of mizoram. There has not been a significant phytochemical report on the plant, and therefore the study has been done

Materials and methods

The plant has been collected from Rawpui village, Serchhip district and Sihphir, Aizawl district. The leaves and stems were dried under shade and then powdered. The powdered samples were extracted with Methanol by hot continuous extraction process using soxhlet apparatus for 72 hours. The crude extract is concentrated and dried using rotary vacuum evaporator and eleven phytochemical tests were performed.

Phytochemical report

Byttneria aspera was the plant under study. Methanol extraction was done for 3 successive batches using soxhlet apparatus and Rotary vacuum evaporator was used for further extraction of the plant compounds. The crude extract was tested by the different tests given below, to check the presence or absence of certain phytochemicals and secondary metabolites.

1. Alkaloids: plant extracts were dissolved individually in 10 % dilute Hydrochloric acid and filtered

- Mayer's test:** Filtrates were treated with mayer's reagent (potassium mercuric Iodide) and formation of a yellow precipitate was observed thereby indicating that the sample is Positive
- Wagner's test:** Filtrates were treated with Wagner's reagent (iodine in potassium Iodide). There was a formation of brown reddish precipitate which indicates that the sample is Positive.

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- c) **Dragendroff's test:** Filtrates were treated with Dragendroff's reagent (solution of potassium Bismuth Iodide). There was a formation of red precipitate which indicates the presence of alkaloids thereby indicating that the sample is Positive
- d) **Hager's test:** Filtrates were treated with Hager's reagent (saturated picric acid solution). Formation of yellow precipitate indicates the presence of alkaloids, therefore the sample is considered to be Positive

Carbohydrates: Plant extracts were dissolved individually in 5 ml distilled water and filtered. The filtrates were used to test for the presence of carbohydrates using the following tests:

- a) **Molisch's test:** Filtrates were treated with 2 drops of alcoholic α -naphthol solution in a test tube. There was a formation of a violet ring at the junction which indicates the presence of carbohydrates, therefore it was POSITIVE.
- b) **Benedict's test:** Filtrates were treated with Benedict's reagent and heated gently, presence of orange red precipitate will indicate the positive reaction, however, there was no formation of orange red precipitation, therefore the test is considered NEGATIVE.
- c) **Fehling's test:** Filtrates were hydrolysed with 10% HCl, neutralised with alkali and heated with Fehling's A & B solutions. Presence of RED precipitate was not detected

Detection of glycosides: Extracts were hydrolysed with dil. HCl and then subjected to test for glycosides.

Modified Borntrager's test: extracts were treated with ferric chloride solution and immersed in boiling water for about 5 minutes. The mixture was cooled and extracted with equal volumes of benzene. The benzene layer was separated and treated with ammonia solution. Formation of rose pink color in the ammoniacal layer indicates the presence of anthranol glycosides.

Legal's test: Plant extracts were treated with sodium nitroprusside in pyridine and sodium hydroxide. Formation of pink to blood red colour indicates the presence of cardiac glycosides, however this sample was found to give a negative result

Detection of saponins

- a. **Froth test:** Plant extracts were diluted with distilled water to 20 ml and this was shaken in a graduated cylinder for 15 minutes. Formation of 1 cm layer of foam indicates the presence of saponins
- b. **Foam test:** 0.5gm of extract was shaken with 2ml of water. If foam persists for 10 mins, it indicates the presence of saponins

Detection of phytosterols

- a. **Salkowski test:** Plant extracts were treated with 5ml chloroform and filtered. The filtrates were treated with few drops of conc. sulphuric acid, shaken and allowed to stand. Appearance of golden yellow ring indicates the positive result however for this plant extract, the plant shows NEGATIVE result.
- b. **Libermann buchard's test:** Plant extracts were treated with chloroform and filtered. The filtrates were treated with few drops of acetic anhydride, boiled and cooled. Concentrated Sulphuric acid was added. There was a formation of bluish green ring at the junction which indicated the presence of phytosterols.

Detection of phenols

Ferric chloride test: Extracts were treated with 3 to 4 drops of ferric chloride solution. Formation of bluish black color was not observed, therefore the test is considered to be negative.

Detection of tannins

Gelatin test: 1% gelatin solution containing sodium chloride was added to the plant sample. There was the formation of white precipitate which indicates the presence of tannins, therefore the test is POSITIVE

Detection of flavonoids

- a. **Alkaline reagent test:** Plant extracts were treated with few drops of 10% sodium hydroxide solution. Formation of intense yellow color which becomes colorless on addition of dilute acid indicates the presence of Flavonoids, therefore the test is POSITIVE.
- b. **Lead acetate test:** Extracts were treated with 3ml of 10% lead acetate solution. Formation of yellow color precipitate indicates the presence of flavonoids therefore the test is POSITIVE.

Detection of proteins and amino acids

- a. **Xanthoproteic test:** The extracts were treated with few drops of concentrated Nitric acid. Formation of yellow color indicates the presence of proteins, thereby giving a POSITIVE result
- b. **Ninhydrin test:** To the plant extract, 0.25% w/v ninhydrin reagent was added and boiled for a few minutes. Formation of blue color is absent therefore the test is considered to be NEGATIVE.

Detection of diterpenes

Copper acetate test: Plant extracts were dissolved in water and treated with 3-4 drops of copper acetate solution. Formation of emerald color indicates the presence of diterpenes.

Results and discussion: The results obtained from the different phytochemical tests performed are as given below:

Table1: Phytochemical screening report of ethno medicinal plant *Buettnera aspera* (zawngluang)

Sl.no	Name of phytochemical test (secondary metabolites)	result	Inference
1.	Alkaloids		
a.	Mayer's test	+	Yellow precipitate
b.	Wagner's test	+	Reddish brown
c.	Dragendroff's test	+	Red precipitate
d.	Hager's test	+	Yellow precipitate
2.	Carbohydrates		
a.	Molisch's test	+	Violet ring
b.	Benedict's test	-	orange-red ppt
c.	Fehlings test		red ppt
3.	Glycosides		
	Modified Borntrager's test	+	Rose pink
4.	Legal's test	-	Pink to blood red
5.	saponins		
a.	Froth test	+	1 cm layer of foam
b.	Foam	+	Foam formation
6.	Phytosterols		
a.	Salkowski's test	-	golden yellow ring
b.	Liebermann buchard's test	+	Bluish green ring
7.	Phenols		
	Ferric chloride	-	Bluish black
8.	Tannins		
	Gelatin	+	White precipitate
9.	Flavonoids		
a.	Alkaline reagent test	+	Intense yellow color
b.	Lead acetate test	+	Yellow precipitate
10.	Proteins and amino acids		
a.	Xanthoproteic test	+	Yellow color
b.	Ninhydrin test	-	Blue color
11.	Diterpenes		
	Copper acetate test	+	Emerald color

Note: + = present in less or minute quantity ++ = present in large quantity
= absence of the metabolite

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