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Preliminary phytochemical investigation of *Combretum albidum* G. Don; An ignored medicinally important liana.

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Abstract
Day by day faith of people on herbal medicine is increases due to the side effect of synthetic drugs, and results into people started looking back to the traditional knowledge of plant for their health care in day to day life. Certain local practitioner, traditional healers uses the decoction of the fruit of *Combretum albidum* G. Don in the treatment of diarrhoea and dysentery, stem barks used in jaundice. So the present study deals with preliminary phytochemical analysis of stem, leaf, flower and fruit using six different solvent. It needful helps for the scientific documentation and standardization of row plant material used in medicine and its worldwide acceptance.

Keywords: *Combretum albidum*, Lianas and Phytochemical analysis.

INTRODUCTION

Plants fulfill the needs of not only human being but also entire animal kingdom, especially due to the presence of diverse bioactive compounds. Lianas are the important growth forms common to the most of the tropical forest it contribute substantially to the diversity of forest provides food and are widely used by the local people mostly for the medicine. (Bongers *et.al.*2002).

The liana *Combretum albidum* G.Don belonging to the family combretaceae. *C. albidum* is commonly known as Pivalvel in Marathi and Buffalo calf in English. It is an extensive woody twiner occupying the canopy of host tree. Leaf opposite, elliptic to elliptic lanceolate narrow at base, flower sessile in short dense in panicles axillary spike, cream white in colour, fruit pale brown with 4 papery transversely striate wings. Its distribution is restricted to the semi evergreen and dry deciduas forest, along the river bank. Decoction of fruit is taken thrice in a day for the treatment of diarrhoea and dysentery (S. Karuppusamy 2007) and stem barks used in jaundice (Sreedhar e.al 2012). This information appreciating ethanobotanical knowledge of the plant an effect has been made in this study to evaluate and characterizing them by screening preliminary phytochemical analysis. The study also useful for the utilization natural flora as the therapeutic agents.

MATERIALS AND METHODS

Combretum albidum G. Don. was collected during the flowering period. It collected from Patur forest ranges, Alegaon forest ranges and Wari hanuman area in Telhara tehsil during January 2010 to April 2010. Which were identified with help of standard floras (Kamble and Pradhan, 1988 and Naik, 1998). The collected plant material was shade dried and packed in air tight polythene bags for the further use.

Extraction of plant materials

The collecting plant materials were washed and shade dried. The dried plant material is powdered using mixer grinder, Preliminary phytochemical analysis was carried out using six solvent according to the polarity i.e. Petroleum ether, Benzene, Chloroform, Acetone, Ethanol and water respectively by Soxhlet method for 18 hr. Preliminary phytochemical screening done using standard procedures to identify constituents, as described by Harborne (1984), Trease and Evans (1979). It involves testing of different classes of compounds. The methods used for detection of various phytochemicals were followed by qualitative chemical test to give general idea regarding the nature of constituents present in crude drug.

Tests for carbohydrates
Fehling's Test

1 ml Fehling's A solution and 1 ml of Fehling's B solution were mixed and boiled for one minute. Now the equal volume of test solution was added to the above mixture. The solution was heated in boiling water bath for 5-10 minutes. First a yellow, then brick red precipitate was observed.

Benedict's test

Equal volumes of Benedict's reagent and test solution were mixed in a test tube. The mixture was heated in boiling water bath for 5 minutes. Solution appeared green showing the presence of reducing sugar.

Molisch's test

Equal volumes of Molisch's reagent and test solution were mixed in a test tube. The mixture was heated in boiling water bath for

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5 minutes. Appearance of violet or purple colour ring showing the presence of reducing sugar.

Tests for proteins:
Biurret Test

To the small quantity of extract 1-2 drops of Biurret reagent was added. Formation of violet colour precipitate showed presence of proteins.

Million's Test

To the small quantity of extract 1-2 drops of Million's reagent was added. Formation of white colour precipitate showed presence of proteins.

Tests for Anthraquinone glycosides
Borntrager's Test

To the 3ml of extract, dil. H_2SO_4 was added. The solution was then boiled and filtered. The filtrate was cooled and to it equal volume of benzene was added. The solution was shaken well and the organic layer was separated. Equal volume of dilute ammonia solution was added to the organic layer. The ammonia layer turned pink showing the presence of glycosides.

Tests for Cardiac glycosides (Keller- Killiani Test)

To the 5ml of extract, 1ml of conc. H_2SO_4, 2ml of Glacial acetic acid and 1 drop of $FeCl^3$ solution was added. Appearance of Brown ring shows the presence of cardiac glycosides.

Tests for Coumarins

To the 2ml of extract 10% NaOH was added and shake well for 5 min shows the yellow colour.

Tests for Quinone

To the 2ml of extract conc. H_2SO_4 was added and shake well for 5 min shows the Red colour.

Test for steroids
Salkowski Test

To 2 ml of extract, 2 ml of chloroform and 2 ml of conc. H_2SO_4 was added. The solution was shaken well. As a result chloroform layer turned red and acid layer showed greenish yellow fluorescence.

Tests for alkaloids
Hager's Test

To the 2-3 ml of filtrate, 1ml of dil. HCl and Hager's reagent was added and shake well. Yellow precipitate was formed showing the presence of alkaloids.

Mayer's Test

To the 2-3 ml of filtrate, 1ml of dil. HCl and Mayer's reagent was added and shake well. Formation of yellow precipitate showed the presence of alkaloids.

Dragendroff's Test

To the 2-3 ml of filtrate, 1ml of dil. HCl and Dragendroff's reagent was added and shake well. Formation of orange-brown precipitate showed the presence of alkaloids.

Wagner's reagent test

To the 2-3 ml of filtrate, 1ml of dil. HCl and Wagner's reagent reagent was added and shake well. Formation of redish-brown precipitate showed the presence of alkaloids.

Tests for flavonoids
With Lead Acetate

To the small quantity of extract lead acetate solution was added. Formation of yellow precipitate showed the presence of flavonoids.

Tests for Tannins and Phenolic compounds
$FeCl_3$ Solution Test

On addition of 5% $FeCl_3$ solution to the extract, deep blue black colour appeared.

Lead Acetate Test

On addition of lead acetate solution to the extract white precipitate appeared.

Test for Saponins
Foam Test

To 1ml extract 20ml distilled water was added and shakes well in measuring cylinder for 15 min. Then 1cm layer of foam was formed. Above phytochemicals analysis will be carried out using standard procedure (Kokate, 1988; Harborne, 1998 and Sadashivan and Manickam, 2005).

RESULTS AND DISCUSSION

In Table 1 the preliminary phytochemical screening of leaf and stem in various extract i.e. petroleum ether, benzene, chloroform, acetone, ethanol and water shows that there is presence of alkaloids, carbohydrate, proteins, cardiacglycoside, saponins, coumarins, tannins, flavonoid & phenolics compounds. The majority of phytoconstituents are found in acetone, ethanol and water extracts. Except phytosterol present only in the leaves in petroleum ether benzene chloroform and aqueous extract. Antraquinoneglycoside present only in the stem in petroleum ether, benzenes and chloroform. Quinone is present in stem only in benzene, acetone and ethanol extract while, fixed oil and fat are totally absent in all extracts.

In Table 2 the preliminary phytochemical screening of flower and fruit in various extract i.e. petroleum ether, benzene, chloroform, acetone, ethanol and water shows that there is presence of alkaloids, carbohydrate, proteins, cardiacglycoside, coumarins, tannins, anthraquinoneglycoside flavonoid & phenolics compounds.

Phytosterol present only in fruit in acetone, ethanol and water extract. Fat and fixed oil detected in fruits. While, saponins and quinone are totally absent in all extracts.

Table 1. Preliminary Phytochemical screening of leaf and stem of *Combretum albidum* G. Don

S. N.	Constituents	Chemical Tests	Extracts											
			P		B		C		A		E		W	
			L	S	L	S	L	S	L	S	L	S	L	S
1	Alkaloids	Hager's Reagent	+	-	-	-	-	-	+	+	+	+	-	-
		Dragendroff's Reagent	-	-	-	-	-	-	+	+	+	-	-	-
		Mayer's Reagent	-	-	-	-	-	-	+	-	+	+	-	-
		Wagners reagent	-	-	-	-	-	-	+	+	+	+	-	+
2	Carbohydrates & Glycosides	Fehling's Reagent	-	-	-	-	+	+	-	-	+	+	+	+
		Benedict's Reagent	+	-	-	-	-	-	+	+	+	+	-	+
		Molisch's Reagent	-	+	+	-	+	-	-	+	-	+	-	-
3	Steroids	Salkowski Reagent	+	-	+	-	+	-	-	-	-	-	+	-
4	Saponin	Foam	-	-	-	-	-	-	+	+	+	+	+	+
5	Phenolics & Tannin	$FeCl_3$ Sol.	+	-	-	-	-	-	+	+	+	+	-	+
		Lead Acetate	-	-	-	-	-	-	+	+	+	+	+	+
6	Fixed oil & Fats	Spot test	-	-	-	-	-	-	-	-	-	-	-	-
7	Proteins	Biurret Reagent	-	-	-	-	-	-	-	-	+	-	+	-
		Million's Reagent	-	-	-		-	-	+	+	+	+	+	+
8	Anthraquinone glycosides	Borntrager's Reagent	-	+	-	+	-	+	-	-	-	-	-	-
9	Cardiac glycosides	Keller-Killiani Reagent	+	+	+	-	+	+	-	+	+	+	+	+
10	Flavonoids	Lead Acetate	-	-	-	-	-	-	-	+	-	+	-	+
		Extract + NH_3	+	+	+	+	+	+	+	+	-	+	+	+
11	Quinone	Extract +Conc. H_2SO_4	-	-	-	+	-	-	-	+	-	+	-	-
12	Coumarins	Extract +10% NaOH	-	-	-	-	-	-	+	+	+	+	+	+

Note : P- Petroleum ether, B- Benzene, C-Chloroform , A- Acetone, E- Ethanol, W- Water, L-Leaves, S-Stem

Table 2. Preliminary Phytochemical screening of Flower and fruit of *Combretum albidum* G. Don

S. N.	Constituents	Chemical Tests	Extracts											
			P		B		C		A		E		W	
			Fl	Fr	Fl	Fr	Fl	Fr	Fl	Fr	Fl	Fr	Fl	Fr
1	Alkaloids	Hager's Reagent	-	-	-	-	-	-	+	+	+	+	-	-
		Dragendroff's Reagent	-	-	-	-	-	-	+	+	+	+	-	-
		Mayer's Reagent	-	-	-	-	-	-	+	+	+	+	-	-
		Wagners reagent	-	+	-	-	-	-	+	+	+	+	+	+
2	Carbohydrates & Glycosides	Fehling's Reagent	-	-	-	-	+	+	+	+	+	+	+	+
		Benedict's Reagent	+	+	+	+	+	-	-	+	-	+	-	-
		Molisch's Reagent	-	-	-	-	-	-	-	-	+	-	+	-
3	Steroids	Salkowski Reagent	-	-	-	-	-	+	-	+	-	+	-	+
4	Saponin	Foam	-	-	-	-	-	-	-	-	-	-	-	-
5	Phenolics & Tannin	$FeCl_3$ Sol.	-	-	-	-	-	-	+	+	+	+	+	+
		Lead Acetate	-	-	-	-	-	-	+	+	+	+	+	+
6	Fixed oil & Fats	Spot test	-	-	-	-	-	-	-	+	-	+	-	+
7	Proteins	Biurret Reagent	-	-	-	-	-	-	-	-	-	-	-	-
		Million's Reagent	-	-	-	-	-	-	+	+	+	+	+	+
8	Anthraquinone glycosides	Borntrager's Reagent	+	+	+	+	+	+	+	-	+	-	-	-
9	Cardiac glycosides	Keller-Killiani Reagent	-	-	-	-	+	+	-	+	+	+	+	+
10	Flavonoids	Lead Acetate	-	-	+	+	-	+	+	+	+	+	+	+
		Extract + NH_3	-	-	+	-	+	-	+	+	+	+	-	+
11	Quinone	Extract +Conc. H_2SO_4	-	-	-	-	-	-	-	-	-	-	-	-
12	Coumarins	Extract +10% NaOH	-	--	-	-	-	+	+	+	+	+	+	+

Note : P- Petroleum ether, B- Benzene, C-Chloroform , A- Acetone, E- Ethanol, W- Water, Fl-flower, Fr-Fruit.

Alkaloids which have anti-inflammatory activity were present in the leaves, stem, flower and fruit. Saponins which have anti-inflammatory and considered as hemotoxic it is present in the leaves and stem. Coumarines were present in the four of the plant part which is precursor for several anticoagulants. Tannins were present in the four of the plant parts which have astringent and detergent properties were also present and can be used against diarrhea (Trease and Evnas, 2002; Bruneton, 1999). There has been an assertion by Trease and Evans (2002) that naturally cardiac glycosides are used for treatment of various diseases associated with the heart such as in controlling supraventicular (atrial) cardiac arrythmias, it also exert a slowing and strengthening effect on failing

heart.(Essiett, U.A *e.al* 2010) The presence of this compound in *Combretum albidum* G. Don could be useful in the treatment of diseases associated with the heart, anti-inflammatory action, anticoagulant, diarrhea and dysentery.

CONCLUSION

This study provides an ethnobotanical data of the medicinal plants used by the local practitioners and traditional healer to cure different diseases. Moreover, this study will promote a practical use of botanicals and must be continued focusing on its pharmacological validation. Further detailed exploration and collection of ethanobotanical information, chemical studies and screening for medicinal properties will provide cost effective and reliable source of medicine for the welfare of humanity. The knowledge received from this investigation will be very useful for researchers in ethnobotany and pharmacology. The observations from the present study need to be validated with pharmaco-chemical studies in order to confirm their effectiveness.

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