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Anti-oxidant activity and microscopic studies of Kumbi stem bark

Manbir Kaur, Rakesh Yadav and Ravi Kumar Dhawan

Abstract
Phyto pharmaceuticals are derived from natural sources and mostly from plants. The method of cultivation, collection and harvesting mutate the concentration of secondary metabolites. So there is need of standardization of these crude drugs. The present study deals with the preliminary screening and microscopic relevance of stem bark of Kumbi. Moreover the ethanolic, hydroalcholic and aqueous extracts are subjected to DPPH analysis and their anti-oxidant potential is determined.

Keywords: Kumbi, powder microscopy, standardization, *Careya arborea*

Introduction
The variation in secondary metabolites (Muhammad *et al*., 2011 and Anna, *et al*., 2011) [4, 5] is due to differences in growth, geographical location, and time of harvesting. Herbal drugs play an important role or act as a vital ingredient in Homeopathic, Ayurvedic, and Naturopathic and in other different systems of medicine. During the past decade, there has been increasing public interest and acceptance of natural therapies worldwide. The developing countries use herbal medicine as their source of primary healthcare (Bodeker *et al*., 2005; Mukherjee, 2002; Bandaranayake *et al*., 2006) [1, 3, 6].
The shortcoming of allopathic medicine are getting more apparent so there is a shift towards the use of medicine of herbal origin globally. Generally, all medicines, whether they are synthetic or of plant origin, should fulfill the basic requirements of being safe and effective (EMEA, 2005; WHO, 2002) [12, 13]. Standardization is a tool of quality control process comprising of a set of standards or inherent characteristics, constant parameters, definitive qualitative and quantitative values that carry an assurance of quality, efficacy, safety and reproducibility.
Careya arborea Roxb. Is a member of Lecythidaceae family and is found in different tropical areas of the world like India, Sri Lanka and Malaya peninsula. (Gupta, 2012 and the ayurvedic pharmacopoeia) [9].
Careya arborea is medium sized deciduous tree with height about 20 meters with handsome spreading crown. Surface of bark is fissured and dark grey in color. Leaves are simple, broadly obovate, tapering towards base. Fruit is drupe, many-seeded, globose to depressesdglobose, crowned by sepals. Seeds have large embryo and obsolete cotyledons nesting in fleshy pulp (Parrotta *et al*., 2001) [18]. The traditional use of bark of *Careya arborea* is to treat tumors, bronchitis, as astringent, antidote to snake venom and skin diseases. In addition, the bark also find useful as demulcent, alexiteric, expectorant, anthelmintic, antipyretic and antipruritic (Kumar *et al*., 2008) [21] and in different conditions such as toothache, wounds, catarrh, dyspepsia, colic, haemorrhoids, intestinal worms, diarrhea and dysentery, leucoderma, epilepsy, abscesses, Bark of the *Careya arborea* chiefly showed the presence of steroids, terpenoids, (Kamal Kumar, 2013) [7] alkaloids, flavonoids (Ragavendran, 2015) [8] and saponins, (Ramanathan, 2016) [11]
Tannins. Pyroligenous acid and other components (Kedare, 1953) [22] are also reported. The other chief constituents present in it are terpenoids, flavonoids, alkaloids, saponins and tannins. (Wadkar, 2008) [20].

Material and Methods
Plant Material
The stem bark of plant is procured from Triputi region of Andhra Pradesh and was authenticated by Dr. K. Madhava Chetty, department of botany at Sri Venkateswara University, Tripura, A.P. The specimen is assigned a voucher no. 6310 was deposited at the herbarium section of departmental museum for reference.

Pharmacognostic Evaluation
Stem was taken for organoleptic and cellular studies. Coarse powder was used to study microscopical features, foaming index and phytochemical investigation. For the microscopical studies, transverse sections of stem was prepared and stained as per standard procedure. The powder microscopy and phytochemical screening were performed according to the method of Khandelwal. Foaming index was found according to the well-established official method and procedure.

Fluorescence Analysis
Powdered bark material was treated with various chemical reagents and exposed to visible, ultraviolet light (Short UV) to study their fluorescence behavior.

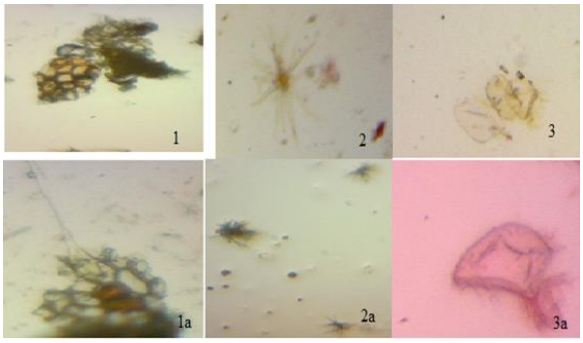
Antioxidant activity (DPPH radical scavenging method)
DPPH solution: DPPH stock solution was prepared by dissolving 0.002 mg of DPPH in 100 ml of methanol. (Vaidyaratnam, 2002) [14] Standard solution: Ascorbic acid was used as a standard free radical scavenger. This was prepared by dissolving 20 mg of ascorbic acid in 20 ml methanol to get 1000ug/ml stock solution. Serial dilutions were then made to get concentrations of 25ug/ml, 50ug/ml, 75ug/ml and 100ug/ml. (Ahmed, 2013) [15]
Test solution: The test compound was prepared in methanol by dissolving 20mg of the test compound (ethyl acetate and chloroform) in 20 ml of methanol to get 1000ug/ml solution. Serial considerations were then made to get concentration of 25ug/ml, 50ug/ml, 75ug/ml and 100ug/ml. (Patel, 2011) [16]
DPPH assay: Different concentration (25ug/ml, 50ug/ml, 75ug/ml and 100ug/ml) of compound and standard separately were prepared in methanol. In clean and labeled test tubes, 2ml of DPPH solution (0.002% in methanol) was mixed with 2ml of different concentrations of compounds and standard separately. The tubes were incubated at room temperature in dark for 30 minutes and then optical density was measured at 517nm using UV- Visible spectrophotometer. The absorbance of DPPH control was also noted. The scavenging activity was calculated using the formula. (Koleva, 2002) [17]

% inhibition= control- test/control*100

Results and Discussion
Organoleptic Characters

Decription	Stem bark	Powdered Stem bark
Colour	Externally dark brown and internally light brown	Pinkish brown
Odour	None	None
Taste	Astringent	Astringent
Shape	Curved	Coarse powder
Surface	Longitudinally wrinkled	……

Powder microscopy



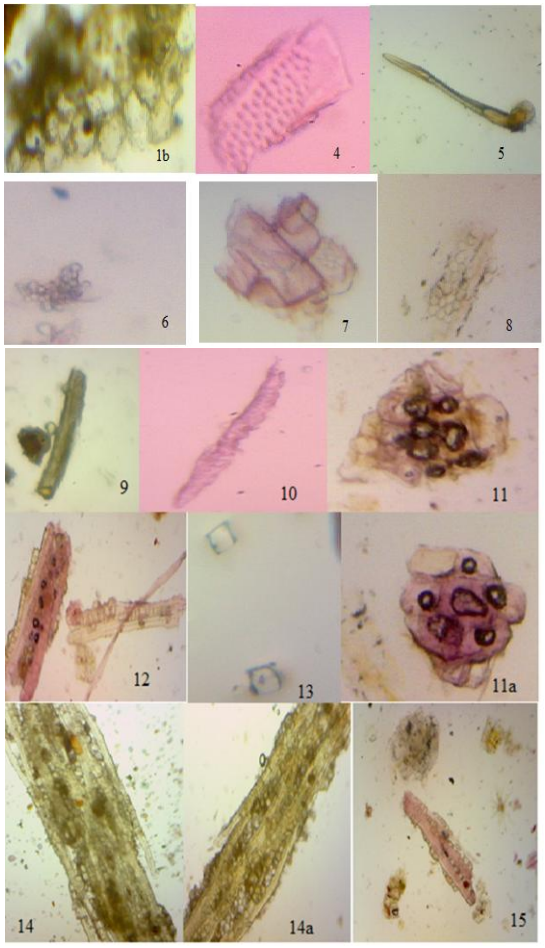


Fig 1: Powder characteristics of bark of *Careya arborea*

1. Diffrent types of cork viz cork in surface view
1a. Cork cells with embedded brownish yellow pigment
1b. cork in sectional view
2, 2a. Acicular crystals
3,3a. Stone cells
4. Perforated conducting vessel
5. Fibre
6. Starch grains
7.Three sided parenchyma with starch grains
8. Polygonal parenchymatous cells
9. Conducting element
10. Sclereids
11, 11a. Collenchyma with stone cells
12. Phloem fibre tangential- medullary rays along with cysal fibres and brown color pigment
13. Prismatic crystals
14, 14 a. Medullary rays embedded in phloem fibres and yellow pigment matter
15. Lignified crystal fibre

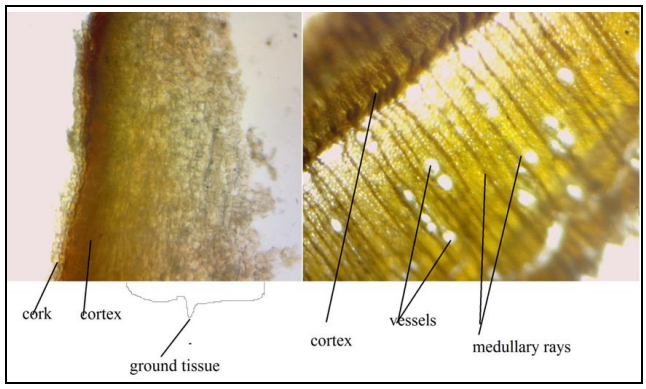


Fig 2: TS of stem bark of *Careya arborea* Rosb.

Foaming index of *Careya arborea* is mentioned in Table 1 and is found to be 142.85

Table 1: Foaming index

Decoction (in ml)	Height of foam (in cm)
1	0.5
2	0.4
3	0.3
4	0.8
5	0.6
6	0.6
7	1
8	0.9
9	0.9
10	0.8

Different extracts show the presence of secondary metabolites as shown in table 2

Table 2: Phytochemical screening

Phytoconstituent	Ethanolic extract	Hydro alcoholic extract	Water extract
Alkaloids	+	+	-
Glycosides	+	+	-
Cardiac glycoside	+	+	-
Carbohydrates	+	+	+
Saponins	-	+	+
Flavonoids	+	+	-
Tannins	+	+	+
Terpinoids	+	+	+

Table 3: Fluorescence analysis

Treatment	Visible light	Under UV light	
		Short wavelength (254 nm)	Long wavelength (365 nm)
Powder	Pinkish grey	Brown	Blackish brown
Powder + 1N NaOH (aq.)	Rusty brown	Dark brown with green tint	Reddish brown
Powder + Ammonia	Light brown	Pale brown	Light brown
Powder + Picric acid	Yellowish brown	Yellowish brown	Brown
Powder + Pet. ether	Light brown	Greenish brown	Light brown
Powder + 50% HCl	Light brown	Greenish brown	Brown
Powder + 50% H_2SO_4	Dark yellowish brown	Dark brown	Yellowish brown
Powder + HCl	Reddish brown	Brown	Brown
Powder + H_2SO_4	Black	Black	Black
Powder + 5% KOH	Reddish brown	Brown	Buff brown

The fluorescence characteristics of the stem bark powder with different chemical reagents are summarized in Table 3.

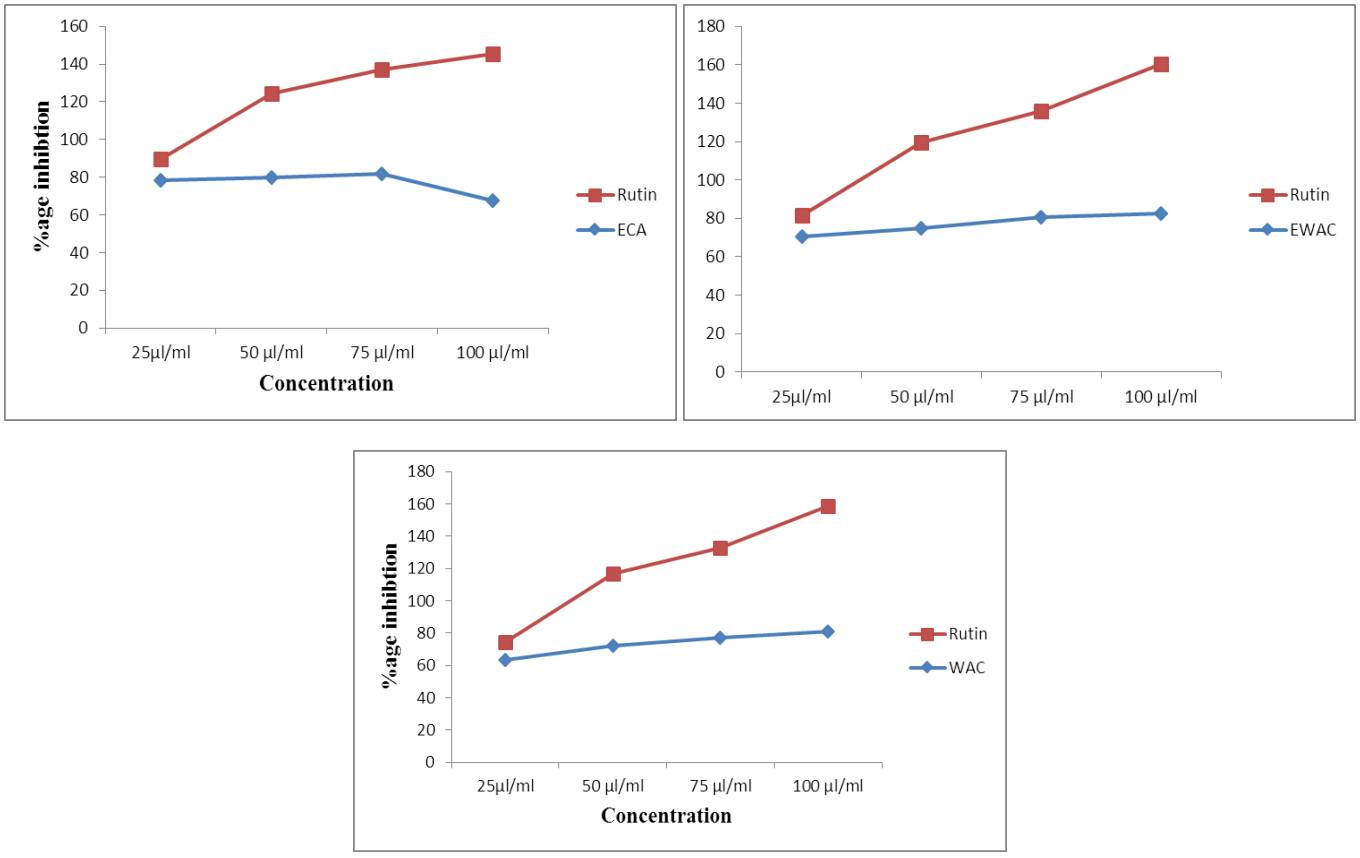


Fig 3: Anti-oxidant activity of different extracts of *Careya arborea*

Conclusion

The plant *Careya arborea* showed the presence of secondary metabolites which is confirmed by performing preliminary phytochemical screening using various reagents. The stem bark of the plant also revealed the existence of different characters which constitute the strengthening and conducting systems of the woody tissue in plants. Moreover different extracts are studied for anti-oxidant activity and resulted in

that hydroalcoholic and aqueous extracts of stem bark showed dose dependent response of percentage inhibition whereas the alcoholic extract does not showed any trends in comparison to standard Rutin.

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