Original Research Article

Ethnomedicobotany And Phytochemical Screening Of *Garcinia pedunculata* Roxb. ex Buch.–Ham From Arunachal Pradesh, India

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Received: December 20, 2015; revised: April 9, 2016; accepted: April 25, 2016

Abstract: *Garcinia pedunculata* Roxb. ex Buch.-Ham commonly known as meba/mibia by the Nyishi tribe of Arunachal Pradesh is a large evergreen tree with fluted trunk with short spreading branches. Leaf mid vein is stout, prominent and lateral veins are distinct. Fruit is globose, dark yellow in colour when ripen. Present study highlights the ethnomedicobotanical use of *G. pedunculata* by the Nyishi community of Papum Pare district of Arunachal Pradesh. The study reveals the use of fruits and leaves in stomach ailments, urinary troubles, jaundice and fever. The mature fruit and tender leaves are also eaten raw and as vegetables. For carrying out the qualitative phytochemical screening, petroleum ether, methanol, chloroform & water extracts were used. Of the four extracts, water and methanol extracts show high affirmative results. Phytochemical screening of fruits, leaves and bark exhibit the presence of bioactive compounds like alkaloids, carbohydrates, saponin, phenolic compounds, proteins along with fixed oils & fats, glycosides and amino acid. Hence the present findings may be helpful to develop conservation and management strategies as well as benefits to pharmaceutical industries.

Key words: Arunachal Pradesh, Ethnomedicobotany, *Garcinia pedunculata*, Nyishi, Phytochemical screening

Introduction

Use of traditional herbal medicines has a greater significance in the present day for the treatment of various ailments because of the higher cost of the modern medical care beyond the reach of poor and side effects of the synthetic drugs. Rural people are still relying on the traditional (knowledge based) medicines which are considered as important components of the primary health care system and are economical, easily available and have little or no side effects in addition to their cultural acceptability (Sinha, 1996; Pal and Shukla, 2003; Dubey *et al.,* 2004). North eastern region of India is having a long history of traditional medicines or folk medicines developed over generations. Arunachal Pradesh is a home to about 28 major tribes and 110 sub-tribes, among which Nyishi is one of the major tribes and predominant community in Papum Pare district of the state with rich indigenous traditional knowledge of the medicinal plant species, the Nyishi people mainly relying on forest and forest resources (Srivastava and Nyishi community, 2008). A number of ethnomedicinal studies have been made by several workers (Jain and Borthakur, 1980; Medhi, 1995; Dutta and Nath, 1999; Kohli, 1999; Sharma Thakur, 1999; Das and Tag, 2006; Sajem and Gosai, 2006; Das *et al.,* 2008; Kalita and Bora, 2008 and Saikia *et al.,* 2010) from various parts of the north east India including Arunachal Pradesh.

Garcinia L. is the largest genus of the family Clusiaceae (Guttiferae), having over 400 species (Cox, 1976). The genus is distributed across the tropical regions of Asia, Africa and Polynesia (Ridley, 1922; Whitmore, 1973). The

genus has about 200 species distributed in the South East Asian region ranging from southern parts of the Thailand and Peninsular Malaysia to Indonesia (Sharma *et al.*, 1993; Mabberley, 1997; Stevens, 2001). In India, occurrence of 35 species of the genus have been reported, among which 15 species are distributed in north east India (Maheshwari, 1964). Present study mainly focuses on *Garcinia pedunculata*, a large evergreen tree with fluted trunk and short spreading branches. Leaf midvein stout, prominent and lateral veins are distinct. Fruit is globose, yellow in colour when ripe (Baruah *et al.,* 2012). Seeds are present 4-8 per fruit and enclosed in a fleshy or succulent aril. The mature fruit is eaten cooked or raw (Patri *et al.,* 2007).

Phytochemicals are non-nutritive plant chemicals that have protective or disease preventive properties. They are natural bioactive compounds found in plant food, leaves or other parts of plants that interacts with nutrients and dietary fiber to protect them. Analysis of phytochemicals revealed the presence and absence of alkaloids, carbohydrates and glycosides, phenolic compounds, saponin, flavanoids, gum and mucilage and proteins (Harborne, 1973; Trease *et al.,* 1989; Andu *et al.,* 2007). Some phytochemical studies on *Garcinia* species have been carried out by several workers (Chowdhury, 2013; Krishnamoorthy *et al.,* 2014; Ramachandran *et al.,* 2014; Jeeva *et al.,*2014) and in other species (Mallikharjuna *et al.*, 2007; De *et al.,* 2010; Saraswathi *et al.,* 2011; Rajan *et al.,* 2011; Katare *et al.,* 2012) are to refer a few. However, no literature is available on the phytochemical screening of *Garcinia* species from Arunachal Pradesh. Therefore, the present study mainly focuses on the ethnobotanical use and its qualitative phytochemical screening.

Materials and methods

Study area

Present ethnobotanical survey was carried out in Papum Pare district of Arunachal Pradesh, located in 27^{0}10´18.85´´ N latitude and 93^{0}42´10.44´´ E longitude, where Nyishi is the predominant tribe of the district possessing rich traditional knowledge of plant utilization particularly herbal medicines. The study was conducted in the villages of Gumto, Doimukh, Rono, Karshingsa, Banderdewa and Kimin (Aibee, Belo–I villages) (Fig. 1), keeping in view the folk use of *G. pedunculata*. Local guides and informers were engaged to locate the species.

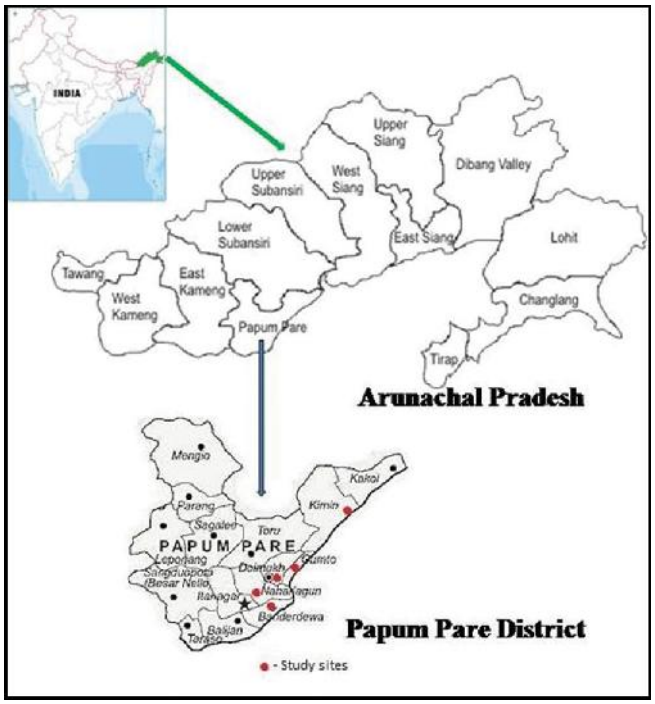

Fig. 1. Map of the study area.

Ethnomedicobotanical survey

The information about the ethnomedicinal uses of *G. pedunculata* was gathered from traditional herbalists and folk medicine practitioners through structured questionnaires. Collections were made following the Participatory Rural Appraisal (PRA) method. Details of the local name, plant parts used and ailments treated with *G. pedunculata* were noted. Specimens were collected and herbarium was prepared following the conventional techniques (Mitra, 1974; Jain and Rao, 1977) and identified using the *Materials for the flora of Arunachal Pradesh* (Hajra *et al.,* 1996) and the nomenclature was updated using mostly the "The Plant List (2013). Version 1.1. Published on the Internet; http://www.theplantlist.org/". Voucher specimens were deposited in the Herbarium of Botany Department, Rajiv Gandhi University, Rono Hills, Doimukh, Arunachal Pradesh.

Preparation of fruit and leaf extracts

Collected fruits, leaves and barks of *G. pedunculata* were air dried and homogenized to powder and stored in airtight bottles. Extracts were prepared by soaking 50g each of the powdered samples in 200ml of each solvent (water, petroleum ether, chloroform and methanol) in a conical flask with repeated shaking at room temperature. After 24h, extracts were filtered through Whatman No.1 filter paper and the filtrates were subjected to qualitative phytochemical tests (Harborne, 1998).

Phytochemical screening

Different extracts were used for preliminary screening of bioactive compounds such as alkaloids (Wagner, Hager and Mayer's test), carbohydrates (Fehling and Molish's test), glycosides (Borntrager's and Legal test), phenolic compounds (Lead acetate, Gelatin and $FeCl_3$ test), Proteins (Millon's and Biuret tests), saponin (Foam test), fixed oils and fats (Spot test and saponification), Gums and mucilages (Alcohol test). Phytochemical screening were done according to Trease and Evans, 1989; Harborne, 1998 and Raman, 2006.

Alkaloids

Wagner test

To 1 ml of the extract, 1 ml of Wagner's reagent is added (iodine in potassium iodide solution). Formation of reddish brown precipitate indicates the presence of alkaloids.

Hager's test

To 1 ml of the extract, 1 ml of Hager's reagent is added (saturated aqueous solution of picric acid). A yellow coloured precipitate indicates the presence of alkaloids.

Mayer's test

To the 1 ml of extract, 1 ml of Mayer's reagent is added (potassium mercuric iodide solution). Whitish yellow or cream coloured precipitate indicates the presence of alkaloids.

Carbohydrates

Fehling test

1 ml Fehling's A and 1ml Fehling's B solutions is mixed and boiled for one minute. Equal volume of test solution were added and heated in boiling water bath for 5-10 min, a yellow precipitate was observed and then brick red precipitate.

Molish's test

To 2-3 ml aqueous extract, few drops of á-naphthol solution is added, shaken and concentrated H_2SO_4 append from sides of the test tube. Observation of a violet ring at the junction of two liquids confirms the result.

Glycosides

Borntrager's test

The test solution is treated with chloroform and the chloroform layer is separated. To this, equal quantity of dilute ammonia solution is added. Colour changes in the ammonical layer shows the presence of glycoside.

Legal's test

To the test solution, 1 ml of the pyridine and few drops of sodium nitroprusside solution are added and then it is made alkaline with sodium hydroxide solution. Colour change shows the presence of glycosides.

Phenolic compounds

To 2-3 ml of extract, few drops of following reagents were added:

a) 5% FeCl3 solution: deep blue-black colour shows the presence of phenolic compounds.

b) Lead acetate solution: white precipitate confirms the presence of phenolic compounds

c) Gelatin solution: white precipitate demonstrate the presence of phenolic compounds

Proteins

Millon's test

3 ml of test solution is mixed with 5 ml of Million's reagent confers white precipitate. Precipitate when warmed turns brick red or dissolves giving red colour shows the presence of proteins.

Biuret test

To 3 ml test solution, 4% NaOH and few drops of 1% CuSO4 solution were added and observed for violet or pink colour which confirms the result.

Table 1. Use pattern of *Garcinia pedunculata* by the Nyishi tribe of Papum Pare district.

Name	Voucher specimen no.	Local Name	Collected from	Parts used	Uses
Garcinia pedunculata Roxb. ex Buch.–Ham	*HGpRGU 03*	Meba/ Mibia	Gumto	Fruits	Used for treating dysentery and diarrhoea. Raw fruits are used to make chutneys.
	HGpRGU 01		Doimukh	Fruits	Juices are used as a cooling agent, in fever and Jaundice.
	HGpRGU 02		Rono	Fruits	Used in dysentery.
	HGpRGU 04		Karshingsa	Fruits	Pulps consumed during indigestion and diarrhoea.
	HGpRGU 05		Kimin	Fruits & leaves	Pulps consumed for treating dysentery. Young tender leaves are used as vegetables.

Saponin

Foam test

The drug extract or dry powder is shaken vigorously with water. Observation of persistent foam shows the presence of saponin.

Fixed oils and fats

Spot test

A drop of concentrated extract is pressed in between two filter papers and kept undisturbed. Oil stain on the paper indicates the presence of oils and fats.

Saponification

Few drops of 0.5N alcoholic potassium hydroxide are added to the extract with few drops of phenolphthalein solution. Later the mixture is heated on water bath for 1-2 hours soap formation indicates the presence of fixed oils and fats in the extracts.

Gum and mucilage

Alcohol test

About 10ml of the extract is slowly added to 25ml of absolute alcohol under constant stirring. Precipitation indicates the presence of gum and mucilage.

Table 2. Qualitative phytochemical analysis of *Garcinia pedunculata* using different solvents.

Constituents	Chemical test	Extracts											
		Aqueous			Petroleum ether			Methanol			Chloroform		
		Fruit	Leaf	Bark	Fruit	Leaf	Bark	Fruit	Leaf	Bark	Fruit	Leaf	Bark
Alkaloids	Mayer's test	+	-	-	+	+	-	+	+	-	+	+	-
	Wagner's test	+	+	+	+	+	+	++	+	+	+	+	+
	Hager's test	-	-	-	-	-	-	+	+	-	-	-	-
Carbohydrates	Molish's test	+	+	+	+	+	-	++	+	-	+	+	-
	Fehling's test	-	+	-	+	-	-	+	+	-	+	-	-
Glycosides	Borntrager's test	-	-	-	-	-	-	-	+	-	-	-	-
	Legal test	-	-	-	+	-	-	+	-	-	-	-	-
Saponin	Foam test	+	+	+	+	+	-	+	+	+	-	+	-
Phenolic compounds	Lead acetate test	+	++	+	-	-	+	+	+	+	+	+	-
	Gelatin test	-	-	-	-	-	-	+	-	+	-	+	-
	$FeCl_3$	+	-	-	-	-	-	+	++	+	-	-	-
Proteins	Millon's test	+	+	++	-	-	-	+	+	-	+	-	-
	Biuret test	-	-	-	-	-	-	-	-	-	-	-	-
Amino acid	Ninhydrin test	-	-	-	-	-	-	+	-	-	-	-	-
Fixed oils and fats	Spot test							+					
	Saponification	+	-	-	-	+	-	-	+	-	-	+	-
Gums and mucilages								++					

+ Moderate, ++ High, - Absent

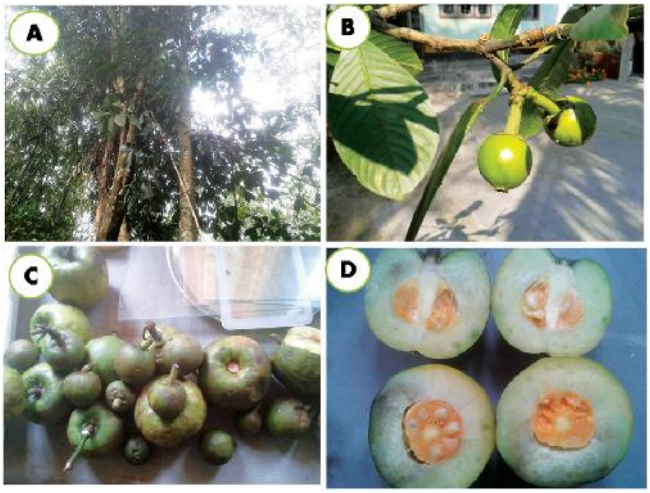

Fig. 2. *Garcinia pedunculata*. (A) A mature tree, (B) Twig with young fruits, (C) Mature fruits and (D) Fruit showing pulp and seeds.

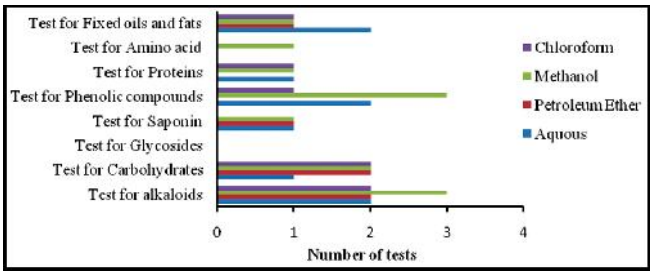

Fig. 3. Screening and distribution of Phytocompounds in the fruit of *G. pedunculata*

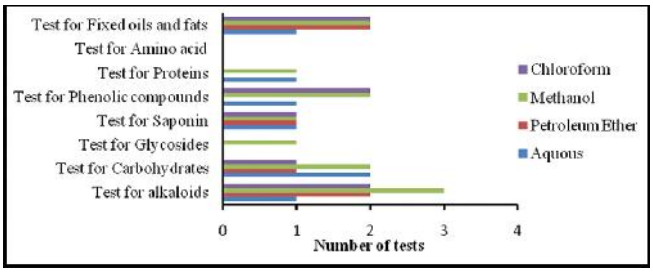

Fig. 4. Screening and distribution of Phytocompounds in the leaf of *G. pedunculata*

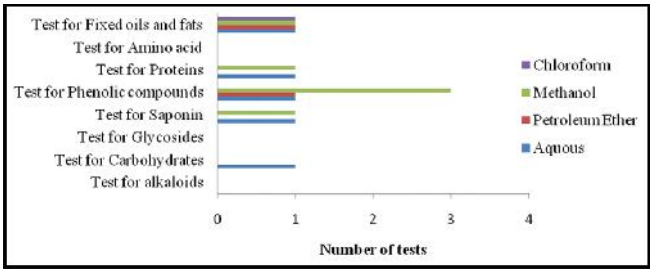

Fig. 5. Screening and distribution of Phytocompounds in the bark of *G. pedunculata*

Results

Local community uses only the leaves and fruits of *Garcinia pedunculata* (Fig. 2), for treating different ailments such as fever, jaundice, indigestion, dysentery and diarrhoea of which dysentery and diarrhoea are most common (Table 1). Though the species is wild-growing, it has been domesticated in backyards of most of the villages for consumption of its fruits as food and medicine. Most people use only the fruit parts and a few use the leaves for curing various ailments. Fruits are edible and used as *"chutney"* and tender leaves are used as vegetables and consumed raw. Details of the species viz. local name, voucher number, parts used, place of collection and ailments treated with the plant parts are presented in Table 1.

Qualitative phytochemical screening reveal the presence of various phytocompounds in higher or in lesser quantity. Water and Methanol extract show high affirmative results among the extracts (Water, Methanol, Chloroform and Petroleum ether). Present phytochemical screening of *G. pedunculata* revealed the occurrence of various phytocompounds viz. alkaloids, carbohydrates, saponin, phenolic compounds and proteins along with fixed oils and fats, glycosides and amino acids (Table 2). For the qualitative phytochemical investigation, three tests each for alkaloids and phenolic compounds, two tests each for carbohydrates, glycosides, proteins, fixed oils and fats plus one test each for gums and mucilages, amino acids and saponin were carried out. From the tests, methanolic extract of the fruit shows the maximum content of alkaloids and phenolic compounds; however, glycoside content is purely absent (Fig. 3). Likewise in leaf extracts, methanolic extract shows the maximum content of alkaloids followed by the other three extracts and amino acids found to be absent (Fig. 4). Secondary metabolites from the bark extracts shows the presence of carbohydrates, saponin, phenolic compounds, proteins along with fixed oils and fats and presence of alkaloids, glycosides and amino acids are found to be negative (Fig. 5).

Discussion

The medicinal plant wealth of the eastern Himalaya plays an important role to the Indian traditional systems of medicine. These medicinal plants have been a source for millions of people in the country. The north eastern region of India harbours more than 8500 species of angiosperms with about 505 of Indian flora of both general and ethnomedicinal plants (Mao and Hyniewta, 2000). In the present study, it was found that most of the tribal people of the Papum Pare district basically the Nyishi tribe are still relying upon the traditional medicinal system, due to the availability of the herbal species proven to be more effective. *G. pedunculata* is used for various ailments such as stomach diseases, urinary troubles, jaundice and fever. Traditional herbal medicine practitioners prescribe a precise dose to the patients based on age, depending on the degree and duration of the ailment, which are indigenous. Knowledge on the use of *G. pedunculata* should be conserved as it would help the pharmaceutical industries and researchers for the exploration of useful phytocompounds (Das *et al*., 2010). A cross-culture study on this species needs further exploration on its ethnobotanical aspects, among the different communities of the north east India.

Regarding the chemical constituents, the essential information of plants is generally provided by the qualitative phytochemical screening of plant extracts. Secondary metabolite studies with the presence of alkaloids, carbohydrates, saponin, phenolic compounds, proteins along with fixed oils & fats, glycosides and amino acid are of great importance in the field of drug research. These classes of bioactive compounds, alkaloids, saponin, flavonoids are known to have activity against pathogens and therefore assist the antimicrobial activities of medicinal plants (Ghosh *et al*., 2010). The curative properties of medicinal plants are possibly due to the presence of various secondary metabolites. The alkaloid and flavonoid content of plant materials has been reported to be a major antioxidant, anti-inflammatory and analgesic active principle. Phenolic compounds like tannins are also potent inhibitors of many hydrolytic enzymes such as proteolytic macerating enzymes used by plant pathogens while saponin is a bioactive antibacterial agent used in hypercholesterolemia, hyperglycemia, antioxidant, anticancer, anti-inflammatory and weight loss etc. (Mandal *et al*., 2005 and Manjunatha, 2006) which were found to be present in the tested plant species. Qualitative tests for different extracts of *G. pedunculata* in the present study showed significant indication about the presence of secondary metabolites. In the phytochemical analysis of *G. pedunculata*, methanolic extract of fruits showed higher affirmative results than the leaves and barks. Presence of these secondary metabolites indicates that *G. pedunculata* may be source of medicine for curing various ailments.

Acknowledgements

Authors are thankful to the local inhabitants and traditional healers of Papum Pare district of the Arunachal Pradesh for sharing valuable information on traditional medicines. We acknowledge the Department of Botany (Center with Potential for Excellence in Biodiversity-II), Rajiv Gandhi University for extending financial support for carrying out the research.

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