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Research Article

PHENOLIC CONTENT, ANTI-OXIDANT AND ANTIMICROBIAL ACTIVITY AND NUTRITIVE VALUE OF YOUNG TWIG OF *PSIDIUM GUAGAVA* LINN. FROM DIBRUGARH, ASSAM

J. CHETIA[1], S. UPADHYAYA[2], D.K. BORA[3] AND L.R. SAIKIA[4]

[1,2,4]**Department of Life sciences, Dibrugarh University, Assam 786004,** [3]**Krishi Vigyan Kendra, AAU, Tinsukia, Assam 786125. Email: sristi_eco@rediffmail.com**

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ABSTRACT

Objective: The present study was an attempt to evaluate the phytochemical constituents, antioxidant and antibacterial activity and nutrient content of young twig of *Psidium guagava* Linn.

Methods: Total phenol and flavonoid content and anti-oxidant activity of the extracts were spectrophotometrically determined; catechol, quercetin and ascorbic acid were taken as standard in case of total phenol, flavonoid content and antioxidant activity respectively. Determination of antimicrobial activity followed by Agar well diffusion method and nutrient content by standard laboratory methods.

Results: The present analysis confirms the presence of tannins, flavonoids, terpenoids, steroids, glycosides, cardiac glycosides, phlobatannin, alkaloids and reducing sugars and absence of saponin and anthraquinone. Total phenol and flavonoid content, and antioxidant activity was found higher in methanol extract than ethanol extract. Positive antibacterial activity was found against *B. cereus* and *S. epidermis* in case of ethanol extracts and against *B. cereus, S. epidermis, E. coli, S. aureus, P. vulgaris,* in case of methanol extracts. In case of antifungal activity, both ethanol and methanol extracts showed positive response against *C. albicans*, while *P.crysogenum* is highly resistant to the extracts. The sample showed a considerable amount of nutritive value (387.76 cal/100gm) also.

Conclusion: The result of the present study provides the scientific basis of use of this plant in traditional health care system.

Keywords:

INTRODUCTION

Plants are the major source of medicines till now. Due to the current global trends of shifting to obtain drugs from plant sources, attention has been given to the medicinal value of herbal remedies.The medicinal value of these plants lies in some chemical substances that produce a definite physiological action on the human body [1].

These plants are source of certain bioactive molecules which act as anti-oxidants and antimicrobial agents [2-5]. *Psidium guajava* Linn. commonly known as Guava belonging to the family Myrtaceae, is a well known traditional medicinal plant used in various indigenous system of medicine. Guavas are rich in dietary fiber, vitamins A and C, folic acid, and the dietary minerals, potassium, copper and manganese. A single *P. guajava* fruit contains about four times the amount of vitamin C as an orange; however, nutrient content varies across guava cultivars [6].

Guavas contain both carotenoids and polyphenols like (+)-gallocatechin [7] guaijaverin, leucocyanidin and amritoside [8] –the major classes of antioxidant pigments – giving them relatively high potential antioxidant value among plant foods [9-11]. Since the 1950s, guavas – particularly the leaves – have been the subject for diverse research on their constituents, pharmacological properties and history in folk medicine [12]. Extracts from apple guava leaves or bark are implicated in therapeutic mechanisms against cancer, bacterial infections, inflammation and pain [13-15].

Essential oils from guava leaves display anti-cancer activity *in vitro* 16]. Guava leaves are used in folk medicine as a remedy for diarrhea [17]; in Trinidad, a tea made from young leaves is used for diarrhea, dysentery and fever [18]; extracts of roots, bark, and leaves are used to treat gastroenteritis, vomiting, diarrhoea, dysentery, wounds, ulcers, toothache, coughs, sore throat, inflamed gums, and a number of other conditions [18].

On the above context, a study was carried out to evaluate the phytochemicals, anti-oxidant and antimicrobial activity and nutrient content of the young twig of *Psidium guagava* collected from Dibrugarh, Assam.

MATERIAL AND METHODS

Sample collection

Young and mature leaves and inflorescence were collected from household premises of Dibrugarh. The materials were shade dried and grounded to fine powder using electric grinder.

Sample extraction

Samples were mecarated separately with water, methanol, ethanol and petroleum ether for 48 hours and filtered through Whatman No 1 filter paper. The filtrate was then evaporated at a constant temperarure (60°C) until a semi dried powder/sticky mass of crude extract was obtained. The crude extract was dissolved in Dimethyl sulphoxide (DMSO) as neutral solvent to make final concentration for biochemical analysis.

Qualitative and quantitative estimation of phytochemicals and anti-oxidants

Qualitative phytochemical analysis of different parts of the plant were done by using standard laboratory methods described by Edeoga et al. [1]; Aja et al. [20]; Ajayi et al. [21]. Quantitative analysis of total phenol and flavonoid were done by the method described by Malik and Singh [22] and Mervat and Hanan [23] respectively. Antioxidant activity of the extracts was done by DPPH and ABTS radical scavenging activity described by Anti-Stanojevic et al. [24] and Re et al. [25] respectively.

Antimicrobial activity

The antimicrobial test was carried out by agar well diffusion method described by Nair et al. [26] using 6mm borer. The activity was determined by measuring the diameter of zone of inhibition (ZOI) exhibited by the extract.

Selected strains for antimicrobial study

Five Gram Positive bacterial strains viz, *Bacillus subtilis* (MTCC 441), *Bacillus cereus* (MTCC 8750), *Staphylococcus aureus* (MTCC 3160), *Staphylococcus epidermis* (MTCC 3615) and *Proteus vulgaris* (MTCC 744); two Gram Negative strains viz, *Escherichia coli* (MTCC 443),

Enterococcus faecalis (MTCC 439) and two fungal strains viz- *Candida albicans* (MTCC 3017) and *Penicillium chrysogenum* (MTCC 947) were used in the study. Strains were obtained from the Microbial Type Culture Collection (MTCC), Institute of Microbial Technology (IMTECH), Chandigarh, India. The reference of bacterial strains were maintained on nutrient agar slants and fungal strains on PDA slants and stored in freeze. Strains were regularly subcultured using nutrient broth for bacterial strains and PDB for fungal strains.

Standard antibiotics

Standard antibiotics viz, Streptomycin (S) 25mcg and Erythromycin (E) 15mcg were taken for bacterial strains and for fungi, Nystatin (NS) 50mcg, Clotrimazole (CC) 10mcg, Ampicillin (AP) 10mcg were employed for comparision of ZOI with sample.

Nutritive value

Moisture, ash and fat content were determined by the method described by Indrayan et al. [27]. Protein content was determined following the method described by Lowry [28] using methanol extract. Finally nutritive value was determined by the following formula:- Nutritive value = 4 x percentage of protein + 9 x percentage of fat + 4 x percentage of Carbohydrate. [27]

Statistical analysis

All the experiments were performed in triplicate and the results were expressed in mean ± S.D.

RESULT AND DISCUSSION

The phytochemical characters of all the samples are summarized in **Table 1**. Presence of tannin, flavonoid, terpenoid, steroid, glycoside, cardiac glycoside, alkaloid, phlobatannin, phenol and reducing sugar and absence of saponin and anthraquinone were recorded in the sample.

Our results are supported by Vanitha et al. [29]; Cho et al. [30]; Narayana et al. [31]; Geidam et al. [32]; on the otherhand, Adeyemi et al. [33] unable to detect alkaloid in the sample. These phytochemicals are playing vital role for the treatment of different types of diseases and therefore they are still used in modern and traditional system of medicine.

Table1: Qualitative phytochemical analysis of *Psidium guagava* twig.

Phytochemicals	Results
Tannin	+
Flavonoid	+
Terpenoid	+
Steroid	+
Glycoside	+
Cardiac glycoside	+
Phlobatannin	+
Alkaloid	+
Reducing sugar	+
Phenol	+
Saponin	-
Anthraquinone	-

+ indicates presence of constituents and – indicate absence of constituents.

Table-2 shows the amounts of radical scavenging activities shown by ethanol and methanol extracts of *P. guajava*. In both the cases, two extracts showed a less amount of antioxidant activity than ascorbic acid. In case of DPPH and ABTS methanol extract showed the higher than the ethanol extract. Uboh et al. [34] said that vitamin C is reported to be one of the antioxidants content of guava leaves' extract.

The DPPH radical scavenging has been widely used to evaluate the free radical scavenging ability of various natural products and has been accepted as a model compound for free radical originating in lipid [35].

Table 2: Antioxidant activities of *P. guagava* twig.

Sample	Antioxidant activity (% inhibition in mg/ml)	
	DPPH radical scavenging activity	ABTS radical scavenging activity
Ethanol extract	80.31± 0.17	79.13± 0.11
Methanol extract	82.38± 0.12	80.36± 0.15
Ascorbic acid	88.20± 0.11	83.00± 0.09

Vanitha et al. [29] showed that methanol, ethanol and water extracts of leaves *of P. guajava* were effective in scavenging the strong free radical DPPH at 100µg concentration and water extract was more potent in scavenging the free radicals followed by ethanol and methanol extracts. According to Jimenez-Escrig et al. [9] guava have relatively high potential antioxidant value among plant foods because of presence of carotenoids and polyphenols which are two major classes of antioxidant pigments.

Table-3 shows the different amount of phenol and flavonoid content of the two extracts. Here also methanol extract showed higher amount of total phenol and flavonoid than ethanol extract.

Table 3: Total phenol and total flavonoid contentof *P. guagava* twig

Samples	Total phenol (mg catechol equivalent/gm dry material)	Total flavonoid (mg quercetin equivalent/gm dry material)
Ethanol extract	7.30± 0.05	2.46± 0.11
Methanol extract	8.50± 0.08	3.86± 0.13

Table-4 shows antibacterial activity of methanol and ethanol extract of the twig of *P. guajava.* Methanol extract against *E. coli* showed the highest zone of inhibition (13mm), followed by methanol extract against *S. epidermis* (12mm), ethanol extract against *B. cereus* (8mm*), S. epidermis* (8mm), *P. vugaris* (8mm) and *S. aureus* (8mm). The essential oil extract of *P. guajava* showed inhibitory activity against *S. aureus* and *Salmonella* spp. [36, 37], a higher concentration of active chemical compounds in essential oils explain their stronger inhibitory action [38, 39]. Joseph *et al*. [37] reported that the essential oil from the leaves of guava exhibited inhibitory effect against *Bacillus cereus, Enterobactor aerogenes* and *Pseudomonas fluorescens*.

In a recent study, it was shown that the *P. guajava* aqueous extract possessed antibacterial activity against *Salmonella typhi* and *pneumoniae*, but no effect on the growth of *Escherichia coli*, *Staphylococcus aureus*, and *Streptococcus fecalis*. The results obtained were found to be encouraging as compared to that of standard antibiotics, though the commercial antibiotics showed a larger inhibitory effect than the methanolic and ethanolic extracts of the twig.

Table 4: Antibacterial activity of the solvent extracts of *P. guajava* twig.

Bacteria	*B. subtilis*	*E. coli*	*S. aureus*	*B. cereus*	*E. faecalis*	*S. epidermis*	*P. vulgaris*
Ethanol extract	-	-	-	8	-	8	-
Methanol extract	10	13	8	-	-	12	10
Strptomycitin (ST) 10mcg	18	-	10	-	-	10	12
Erythromycin(E) 15mcg	32	30	28	30	12	48	12

*- No activity. Zone of inhibition includes the diameter of well (6mm).

Table 5: Antifungal activity of the solvent extracts of *P. guajava* twig.

Test sample	Diameter of inhibition of zone (mm)	
	C.albicans	*P.crysogenum*
Ethanol extract	8	-
Methanol extract	10	-
Nystatin (NS) 50mcg	-	24
Clotrimazole (CC) 10mcg	11	32
Amicillin (AP) 10 mcg	-	46

*- no activity. Zone of inhibition includes the diameter of the well (6mm)

Table-5 shows antifungal activity of methanol and ethanol extract of the plant. In the present study both these two extract showed inhibitory activity against *C. albicans*, but no activity against *P. chrysogenum*; Metwally et al. [40] also reported inhibition of *P. guajava* leaf ethanol extract against *C. albicans.*

Table-6 shows the nutrient content of twig of *P.guajava* which is a considerable amount (387.76 cal/100gm). The result of the present study are encouraging as the plant tested showed the presence of phytochemicals and considerable anti-oxidant and antimicrobial activity, and having good amount of nutrient content.

Therefore, this study provides scientific basis of the use of this plant extracts in traditional health care system.

Table 6: Nutrient content of *P.guajava* twig.

Sample ↓	Ash (%)	Moisture content (%)	Crude fat (%)	Protein (%)	Carbohydrate (%)	Nutritive value (cal/100 g)
Twig	3.77	9.29	8.0	28.5	50.44	387.76

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