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Antimicrobial and Phytochemical Investigation of the Leaves of *Carica papaya* L., *Cynodon dactylon* (L.) Pers., *Euphorbia hirta* L., *Melia azedarach* L. and *Psidium guajava* L.

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Abstract

Carica papaya L., *Cynodon dactylon* (L.) Pers, *Euphorbia hirta* L., *Melia azedarach* L., *Psidium guajava* L. belong to 5 different families, *viz*., Caricaceae, Gramineae (Poaceae), Euphorbiaceae, Meliaceae and Myrtaceae. The plant leaves contain a number of medicinally important compounds. In the present study, we evaluated the phytochemical analysis for the quantity of amino acids, a amylase, ß- amylase, carbohydrate, glutamine, protein, proline, phenolic compounds and the presence of various secondary metabolites such as alkaloids, anthroquinone, catachol, flavonoids, phenols, saponins, steriods, triterpenoids, tannins. The antibacterial activity of the leaf extracts of *Carica papaya, Cynodon dactylon*, *Euphorbia hirta*, *Melia azedarach* and *Psidium guajava* against pathogenic bacteria like gram positive (*Bacillus subtilis, Pseudomonas aeruginosa* and *Staphylococcus aureus*) and gram negative (*Escherichia coli* and *Klebsiella pneumoniae)* bacteria by *invitro* agar well diffusion method. The plants aqueous leaf extracts showed pronounced inhibition than chloroform leaf extracts. Leaf extracts showed more inhibitory action on *Bacillus subtilis, Staphylococcus aureus, Pseudomonas aeruginosa*, *Escherichia coli* and *Klebsiella pneumoniae*.

Introduction

In recent years, the growing demand for herbal product has led to a quantum jump in volume of plant materials traded within and across the countries. In recent years, Secondary plantmetabolites (Phytochemicals), previously with unknown pharmacological activities, has been extensively investigated as a source of medicinal agents (Krishnaraju *et al*., 2005). Thus it is anticipated that phytochemicals with adequate antibacterial efficacy will be used for the treatment of the bacterial infections (Balandrin *et al*., 1985).

Such a fact is cause for concern, because of the number of patients in hospitals who have suppressed immunity, and due to new bacterial strains, which are multi-resistant. Consequently, new infections can occur in hospitals resulting in high mortality.

Nature has been a source of medicinal agents since times immemorial. The importance of herbs in the management of human ailments cannot be over emphasized. It is clear that the plant kingdom harbors an inexhaustible source of active ingredients invaluable in the management of many intractable diseases. Furthermore, the active components of herbal remedies have the advantage of being combined with many other substances that appear to be inactive. However, these complementary components give the plant as a whole a safety and efficiency

much superior to that of its isolated and pure active components (Shariff, 2001).

The screening of plant extracts and plant products for antimicrobial activity has shown that higher plants represent a potential source of novel antibiotic prototypes (Afolayan, 2003).

DESCRIPTION OF PLANTS USED IN THE PRESENT STUDY

Botanical Name : ***Carica papaya* L.**
Family : Caricaceae
Description : Erect, fast growing, usually unbranched tree or shrub, 7-8 m tall, with copious latex, trunk about 20 cm in diameter.
Distribution : Throughout Asia, Papaya has been carried throughout the tropics.
Parts used Leaves, fruit, root, seed, latex.
Uses : Cancer, tumors, corns, nervous pains, piles and yaws. elephantoid growths.

Botancial Name : ***Cynodon dactylon* (L.) Pers**
Family : Gramineae (Poaceae)
Description : Perennial grass, very variable, with long rapid – growing, creeping runner or stolons.
Distribution : Throughout Asia.
Parts used : Aerial parts.
Uses : antiseptic, aperient, astringent, cyanogenetic, demulcent, depurative.

Botanical Name : ***Euphorbia hirta* L.**
Family : Euphorbiaceae
Description : Small, annual herb, that grows 30-40 cm in hight, the stem is slender and often reddish in colour.
Distribution : It is widely distributed in all over the world.
Parts used : Aerial parts.

Uses : Anodyne, Antiasthmatic, antipruritic, carminative, depurative.

Botanical Name : ***Melia azedarach* L.**
Family : Meliaceae
Description : Deciduous tree, leaves alternate, 2-pinnately divided with toothed.
Distribution : Throughout Asia.
Parts used : Leaves, bark, fruit, seed.
Uses : To cure stomach irritation, vomiting, bloody diarrhoea.

Botanical Name : ***Psidium guajava* L.**
Family : Myrtaceae
Description : Evergreen shrub or small tree to 9m tall, with scaly greenish – brown bark and young branches 4-angled.
Distribution : Throughout Asia.
Parts used : the whole guava plant is used.
Uses : To cure diarrhoea, dysentery, stomach upsets and regulate menstrual periods.

MATERIAL AND METHODS

Preparation of leaf extracts

Apparently healthy plant leaves were collected, washed thoroughly in tap water and dried in room temperature for 15 days. The dried 20 g leaves were powdered and soaked separately in 100 ml water and chloroform by keeping it in a shaker for 3 days. The extracts were filtered through cheesecloth and the extracts were reduced to 10% of its original volume. The organic solvent filtrates were concentrated in vacuum using a rotary evaporator, while aqueous

extract was dried using water bath.

Phytochemical Screening of Leaf Extracts

The phytochemical components of the *Carica papaya, Cynodon dactylon*, *Euphorbia hirta*, *Melia azedarach* and *Psidium guajava* leaves were screened by using the methods of Brindha et al., (1977). The components quantitatevely analysed were amino acids, a- amylase, ß- amylase, carbohydrate, glutamine, protein, proline and phenolic compounds. The components qualitatively analysed were alkaloids, anthroquinone, catachol, flavonoids, phenols, saponins, steriods, triterpenoids and tannins.

Inoculums

The test micro- organisms, gram positive (*Bacillus subtilis*, *Pseudomonas aeruginosa* and *Staphylococcus aureus*) and gram negative (*Escherichia coli* and *Klebsiella pneumoniae*) bacteria were obtained from MTCC, Chandigar. The organisms were innoculated into NB (Nutrient Broth) medium, (0.5% Peptone, 0.5% Sodium chloride, 0.15% Yeast extract; pH 7.4) and incubated at 37^{o}C for overnight.

The bacterial cells were harvested by centrifuging at 5000 g for 15 min. The pellet formed was washed twice with PBS (Phosphate Buffer Saline), (10 mM Sodium Chloride, ph 7.4) and the cells were counted by hemocytometer. The bacterial cells were diluted to approximately 10^5 CFU ml before use (Owais et al., 2005).

The antibacterial activity of the leaf extracts was determined using agar well diffusion method by Perez et al., (1990). Nutrient agar was inoculated with the given microorganisms by spreading the bacterial inoculums on the media. Wells (5 mm diameter) were punched in the agar. Then 3 different concentration of plant extract viz., 25µl, 50µl, 75µl, were added to the wells separately. The plates were incubated at 37^{o}C for 18 hours and the antibacterial activity was assessed by measuring the diameter of the zone of inhibition.

RESULTS

Phytochemical screening of leaf extracts

Table 1. Quantitative analysis of leaf samples of certain medicinal plants[a] (mg/g).

Phyto Constituents	**Name of the Plants**				
	Carica papaya	***Cynodon dactylon***	***Euphorbia hirta***	***Melia azedarach***	***Psidium guajava***
Amino acid	0.94	0.87	0.74	0.89	0.97
µ-Amylase	0.68	0.62	0.58	0.64	0.65
b-Amylase	0.68	0.62	0.58	0.64	0.68
Carbohydrate	1.84	1.32	1.72	1.62	1.86
Glutamine	0.21	0.17	0.18	0.19	0.23
Phenols	0.24	0.20	0.17	0.18	0.21
Proline	0.31	0.27	0.14	0.17	0.24
Protein	1.82	1.52	1.61	1.72	1.92

[a]Values are mean of triplicate determinations on dry weight basis.

Table 2. Qualitative analysis of leaf samples of certain medicinal plants (mg /g).

Phytochemicals	Name of the plants				
	Carica papaya	***Cynodon dactylon***	***Euphorbia hirta***	***Melia azedarach***	***Psidium guajava***
Alkaloids	+	+	-	+	-
Anthroquinone	+	+	+	+	+
Catachols	-	-	-	-	+
Flavonoids	+	+	+	+	+
Phenolic compounds	-	-	+	+	+
Saponins	+	+	+	+	+
Steriods	+	+	+	+	+
Tannins	+	+	+	+	+
Triterpenoids	+	+	-	+	-

[a]Values are mean of triplicate determinations on dry weight basis.

Antibacterial activity of leaf extracts

The antibacterial activity of the *Carica papaya, Cynodon dactylon, Euphorbia hirta, Melia azedarach* and *Psidium guajava* leaves were assessed using the agar well diffusion method by measuring the diameter of growth inhibition zones with 25, 50, 75 µl of aqueous and solvent leaf extracts (Table 3,4 and 5).

The results showed that aqueous and chloroform leaf extracts possesses antibacterial activity against tested gram positive (*Bacillus subtilis, Pseudomonas aeruginosa* and *Staphylococcus aureus*) and gram negative (*Escherichia coli* and *Klebsiella pneumoniae*) bacteria. The extracts of *Carica papaya, Cynodon dactylon, Euphorbia hirta, Melia azedarach and Psidium guajava* in aqueous and solvent (Concentration of 75µl /ml) exhibit relatively higher zone of inhibition compare than concentration 25 and 50µl/ml. The *Bacillus subtilis, Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa* and *Staphylococcus aureus* were resistant to aqueous leaf extracts of *Cynodon dactylon* and chloroform leaf extract of *Carica papaya*. In present study, Aqueous extract of *Carica papaya* exhibit higher zone of inhibition compared to chloroform extract.

The growth of bacteria was suppressed by phytochemical compounds of chloroform and aqueous extracts of all the plants with different magnitudes. *Carica papaya* possess higher antibacterial activity among the tested plants. This was followed by *Cynodon dactylon, Euphorbia hirta, Psidium guajava* and *Melia azedarach*.

Table 3. Zone of inhibition in mm (25 µl concentration).

Extracts	Name of the plants	***Bacillus subtilis***	***Escherichia coli***	***Klebsiella pneumoniae***	***Pseudomonas aeruginosa***	***Staphylococcus aureus***
Aqueous	*Carica papaya*	3	3.4	3.2	3.4	3.6
	Cynodon dactylon	-	-	-	-	-
	Euphorbia hirta	3	3.4	3	4	4
	Melia azedarach	3	2.8	3.4	3.4	3.4
	Psidium guajava	2.8	3.2	3.2	3.2	3.2
Solvent	*Carica papaya*	-	-	-	-	-
	Cynodon dactylon	2.8	2.6	2.6	3	3
	Euphorbia hirta	2.5	2.4	2.4	2.6	2.6
	Melia azedarach	2.2	2	2.4	2.4	2.4
	Psidium guajava	2.4	2.8	2.6	2.4	2.4

Table 4. Zone of inhibition in mm (50 µl concentration).

Extracts	Name of the plants	*Bacillus subtilis*	*Escherichia coli*	*Klebsiella pneumoniae*	*Pseudomonas aeruginosa*	*Staphylococcus aureus*
Aqueous	*Carica papaya*	3.6	3.8	3.4	3.8	3.6
	Cynodon dactylon	-	-	-	-	-
	Euphorbia hirta	4	3.4	3	4	4
	Melia azedarach	3.2	3.2	3.4	3.4	3.4
	Psidium guajava	3.6	3.8	3.4	3.2	4
Solvent	*Carica papaya*	-	-	-	-	-
	Cynodon dactylon	3.6	3	2.8	3	3.4
	Euphorbia hirta	3.2	2.4	2.6	2.8	2.6
	Melia azedarach	2.6	2.2	2.6	2.6	2.4
	Psidium guajava	2.8	2.8	2.6	3	2.6

Table 5. Zone of inhibition in mm (75 µl concentration).

Extracts	Name of the plants	*Bacillus subtilis*	*Escherichia coli*	*Klebsiella pneumoniae*	*Pseudomonas aeruginosa*	*Staphylococcus aureus*
Aqueous	*Carica papaya*	4.2	4	4	3.8	4.4
	Cynodon dactylon	-	-	-	-	-
	Euphorbia hirta	4	3.4	3.4	4	4
	Melia azedarach	3.4	3.6	3.6	3.6	3.8
	Psidium guajava	3.8	3.8	3.6	3.6	4.2
Solvent	*Carica papaya*	-	-	-	-	-
	Cynodon dactylon	3.8	3.8	4	3.6	4
	Euphorbia hirta	3.4	2.6	3	3	2.6
	Melia azedarach	2.8	2.4	2.6	3	2.4
	Psidium guajava	3.6	3.6	2.8	3	3

DISCUSSION

The presence of antibacterial substances in the higher plants is well established (Srinivasan, 2001). Plants have provided a source of inspiration for novel drug compounds as plants derived medicines have made significant contribution towards human health. Phytomedicine can be used for the treatment of diseases as is done in case of Unani and Ayurvedic system of medicines or it can be the base for the development of a medicine, a natural blueprint for the development of a drug (Didry *et al*., 1998).

Successive isolation of botanical compounds from plant material is largely dependent on the type of solvent

used in the extraction procedure. The traditional healers use primarily water as the solvent but we found in this study the plant extracts by chloroform provided less consistent antimicrobial activity compared to those extracted by water. The results of antibacterial activity of all the 5 plants against the investigated bacterial strains are shown in Table (3-5).

The present study the extracts from *Carica papaya, Cynodon dactylon, Euphorbia hirta, Melia azedarach* and *Psidium guajava* presented antimicrobial activity to the test organism. The extracts from *Carica papaya* presented the highest activities. They were able to inhibit 60% (types of microorganism) of interest, respectively. Moreover, they also had the highest activity rate against antibiotic resistant bacteria. One of the microorganism that showed susceptivility to this extracts was *Pseudomonas aeruginosa*. Some of the extracts of phytochemicals tested were active. Such results were more resistant to environment conditions.

The higher resistance of gram negative bacteria to plant extracts has previously been documented and related to thick murein layer in their outer membrane, which prevents the entry of inhibitor substances into the cell (Martin, 1995; Brantner *et al.,* 1996; Palombo and Semple, 2001; Tortora *et al.,* 2001; Matu and Van Staden, 2003). Similarly, our results indicated that the antibacterial activities of the extracts were more pronounced on Gram- positive than on Gram –negative bacteria.

In this study, the results obtained indicated that the aqueous and chloroform extracts of the *Carica papaya, Cynodon dactylon, Euphorbia hirta, Melia azedarach* and *Psidium guajava* inhibited the growth of the tested microorganisms viz., *Bacillus subtilis, Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa* and *Staphylococcus aureus*. This, therefore, shows that the extract contains substances that can inhibit the growth of some microorganisms. Other workers have also shown that extracts of some plants inhibited the growth of various microorganisms at different concentrations (Akujobi *et al*., 2004; Esimone *et al.,* 1998; Nweze *et al.,* 2004; Ntiejumokwu and Alemika, 1991;). The observed antibacterial effects on the isolates is believed to be due to the presence of alkaloids, tannins and flavonoids which have been shown to posses antibacterial properties (Cowan, 1999; Draughon, 2004). Some workers have also attributed their observed antimicrobial effects of plant extracts to the presence of these Secondary metabolites. (Nweze *et al*., 2004). Some workers have also identified tannins, flavonoids and alkaloids in the extracts of some medicinal plant (Esimone *et al*., 1998).

Alternatively, the passage of the active compound through the gram negative cell wall may be inhibited. It is though that observed differences may result from the doses used in this study. In addition, microorganisms show variable sensitivity to chemical substances related to different resistance levels between strains (Cetin and Gurler, 1989).

There fore, our results revealed the importance of plant extracts when associated with antibiotics, to control resistant bacteria, which are becoming a threat to human health. Further more, in a few cases, these plant extracts were active against antibiotic resistant bacteria under very low concentration, thus minimizing the possible toxic effects.

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