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RESEARCH ARTICLE

A COMPARATIVE STUDY ON THE *IN VITRO* ANTI-INFLAMMATORY AND ANTIBACTERIAL ACTIVITIES IN THE LEAF EXTRACTS OF *COSTUS IGNEUS AND MANGIFERA INDICA*

***Khoushika Raajshree, R. and Chitra, P.**

Department of Biochemistry, Sri Ramakrishna College of Arts and Science for Women, Coimbatore, Tamilnadu, India

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ABSTRACT

Inflammation is a bodily response to injury, infection or destruction characterised by heat, redness, pain, swelling and it is a normal protective response to tissue injury caused by physical trauma, noxious chemical or microbial agents. Infectious diseases caused by bacteria can become a threat to public health in this world. The rich wealth of plant kingdom represents a novel source of compounds with significant activities. Leaves of *Costus igneus* and *Mangifera indica* were subjected to qualitative tests and quantitative total phenolic content assay. Of all the extracts, the *in vitro* anti-inflammatory and antibacterial activity was found to be maximum in ethanol extract of *Mangifera indica*. Therefore, the plant source leads the way to treat bacterial infections and related inflammations which underlies almost in all diseased conditions.

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INTRODUCTION

In Indian scenario, World Health Organization (WHO) estimates about 70-80% of Indians depend on Indian system of medicine like Unani, Siddha, and Ayurvedha (Gupta and Shaw, 2009). Traditional use of herbal medicine is usually an integral part of culture around the world, which has been used in medical practice for thousands of years and has made a great contribution for maintaining human health before spread of modern science (Verma and Singh, 2008). The emerging importance of biologically active medicinal plants and their constituents as possible therapeutic measures has become a subject of active scientific investigation. It is likely that in future safe and effective medicines will be developed from medicinal plants to treat various degenerative diseases. Many pharmaceutical companies show interest in plant derived drugs mainly due to the current widespread belief that 'Green Medicine' is safe and more dependable than the costly synthetic drugs, which have adverse side effects (Nikhal *et al.,* 2010). Inflammation is a bodily response to injury, infection or destruction characterised by heat, redness, pain, swelling and disturbed physiological functions. It is a normal protective response to tissue injury caused by physical trauma, noxious chemical or microbial agents. It is the body response to inactivate or destroy the invading organisms, to remove the irritants and set the stage for tissue repair. It is triggered by the release of chemical mediators from injured tissue and migrating cells (Tripathi, 2008). It is an adaptive host defensive mechanism against infection or injury. It is a complex local response to foreign substances resulting in fever. Inflammation underlies almost in all diseased conditions and inadequate resolution of these inflammatory responses often leads to cancer (Rathisre *et al*., 2013). The commonly used drug for management of inflammatory conditions are non-steroidal anti-inflammatory drugs, which have several adverse effects especially gastric irritation leading to formation of gastric ulcers. These drugs have potent activity; they have a number of severe adverse effects such as gastrointestinal disturbances and body fat redistribution. Various medicinal plants provide relief from symptoms comparable to that obtained from allopathic medicines. The rich wealth of plant kingdom can represent a novel source of newer compounds with significant anti-inflammatory activities. Herbal drugs are playing major role in the world because of their safety, efficacy and cost effectiveness. In this scenario, use of plant derived products to treat inflammation and related condition becomes a viable and valid approach (Saleem *et al*., 2010). Infectious disease can become a threat to public health in this

****Corresponding author: Khoushika Raajshree, R.***
Department of Biochemistry, Sri Ramakrishna College of Arts and Science for Women, Coimbatore, Tamilnadu, India.

world. The use of medicinal plants for the treatment of various diseases is an old practice in most countries and it still offers an enormous potential source of new anti-infective agents. Although ancient civilization recognized the antiseptic or antibacterial potential of many plant extracts, they failed to document the preservative and curative effects of plant extracts (Arumugam *et al*., 2009). Medicinal plants are considerably useful and economically essential. They contain active constituents that are used in the treatment of many human diseases. The plant extracts have been developed and proposed for use as antibacterial substances (Del-Campo *et al*., 2000). The antibacterial activities of medicinal plants can be attributed to the secondary metabolites such as alkaloids, flavonoids, tannins, terpenoids that are present in these plants. In recent years, secondary plant metabolites (Phytochemicals), previously with unknown pharmacological activities have been intensively investigated as a source of medicinal plants. Thus, it is anticipated that phytochemicals with adequate bacterial efficacy will be used for the bacterial infections. Since, man has used various parts of plants in the treatment and prevention of various ailments (Sher, 2009).

Costus igneus commonly known as fiery costus or Insulin plant is native to South and Central America. This is a recent introduction to India from America as an herbal cure for diabetes and hence commonly called as 'insulin plant' (Jose and Reddy, 2010). *Costus igneus* leaves have been proven to possess various pharmacological activities. *Mangifera indica* (Anacardiaceae) is a tree, distributed in rural and semi urban parts of the India. It is one of the most important tropical plants marketed in the world (Ross, 1999). *Magnifera indica* is a large evergreen tree in the anacardiaceae family that grows to a height of 10-45 m, dome shaped with dense foliage, typically heavy branched from a stout trunk. Phytochemical research from different parts of *M. indica* has demonstrated the presence of phenolic constituents, triterpenes, flavonoids, phytosterol, and polyphenols (Singh *et al*., 2004). Thus the current study is focused to evaluate and compare the anti-inflammatory and antibacterial activities in the leaf extracts of *Mangifera indica* and *Costus igneus* by *invitro* method.

MATERIALS AND METHODS

Collection and preparation of plant materials

Healthy fresh leaves of *Mangifera indica* and *Costus igneus* were collected from the nearby areas of Coimbatore district. The leaves were rinsed with distilled water and dried at room temperature under well ventilated shade for 10 days. The dried leaves were powdered and stored in air-tight container for further analysis.

Extraction of plant material

The powdered leaves were extracted in various solvents, viz hexane, ethyl acetate and ethanol (Gayathri and Jeyanthi, 2013). One part of the powdered leaves were macerated in three parts of hexane, ethyl acetate and ethanol separately and kept for 24 hours at 37°C. Filtered and collected the solvents. The solvents were evaporated to obtain the hexane, ethyl acetate and ethanol extracts.

Qualitative analysis

Phytochemical tests

The methods described by Trease and Evans, 1989 and Abalaka *et al*., 2011 were used for screening of phytochemicals like tannin, saponin, flavonoids, phenols, cardiac glycosides, terpenoids, steroids, phytosteroids, phlobatannins, alkaloids and carbohydrates.

Quantitative analysis

Estimation of total phenolic content

Total phenolic content of the extracts were assessed according to the Folin–Ciocalteau method (Slinkard and Singleton, 1977) with some modifications. Briefly, 0.1 ml of the extracts with varying concentrations (200, 600 and 1000µg/ml), 1.9 ml distilled water and 1.0 ml of Folin–Ciocalteau's reagent were seeded in a tube, and then 1.0 ml of 100 g/l Sodium carbonate was added. The reaction mixture was incubated at 25°C for 2 hours and the absorbance of the mixture was read at 765 nm. The readings were taken in triplicates. The total phenolic content of sample was expressed as mg of catechol equivalents per gram of extract.

Amount TPC = Sample OD/Standard OD * Respective Amount of extract

Where, Sample OD refers to optical density of sample and Standard OD refers to optical density of standard.

Invitro antioxidant activity

DPPH free radical scavenging assay

The ability of the extracts to annihilate the DPPH radical (2, 2-diphenyl-1-picrylhydrazyl) was investigated by the method described by Blois, 1958. Stock solution of extracts were prepared to the concentration of 10 mg/ml. Different concentration of the extract (200, 600 and 1000 µg) of extracts were added at an equal volume to 1.0 ml of methanolic solution of DPPH (0.1mM). The reaction mixture was incubated for 30 minutes at room temperature and the absorbance was recorded at 517 nm. Ascorbic acid was used as standard. The readings were taken in triplicates. The annihilation activity of free radicals was calculated in % inhibition according to the following formula,

% of Inhibition = (Abs of control – Abs of sample)/ Abs of control * 100

Where, Abs control is the absorbance of the control reaction (containing all reagents except the test sample) and Abs sample is the absorbance of the test sample.

Invitro anti-inflammatory activity

Inhibition of albumin denaturation method

Method of Elias and Rao, 1988 was followed with minor modifications for the inhibition of albumin denaturation

method. The reaction mixture consisted of 1.0 ml test extracts at different concentrations (250,500,750,1000µg/ml) and 1% aqueous solution of bovine albumin fraction. pH of the reaction mixture was adjusted to 6.5 using small amount of 1N Hydrochloric acid. The samples were incubated at 37˚C for 20 min and then heated at 57˚C for 20 min. After cooling the samples, the turbidity was measured colorimetrically at 660 nm. The readings were taken in triplicates. Percent inhibition of protein denaturation was calculated as follows:

% of Inhibition = (Abs of control – Abs of sample)/Abs of control * 100

Where, Abs control is the absorbance of the control reaction (containing all reagents except the test sample) and Abs sample is the absorbance of the test sample.

Invitro antibacterial activity

Agar well diffusion method

Antibacterial activity of different extracts were evaluated by the agar well diffusion method Murray *et al*., 1995 and modified by Olurinola, 1996. Nutrient agar (NA) was swabbed (sterile cotton swabs) with 8 hour old - broth culture of respective gram positive and gram negative bacteria. Wells were made in each of these plates using sterile cork borer. Stock solution of each plant extract was prepared at a concentration of 1 mg/ml of different plant extracts viz. Hexane, ethyl acetate and Ethanol. About 100 µl of plant solvent extracts were added sterile syringe into the wells and allowed to diffuse at room temperature for 2 hours. Gentamycin was used as positive control and distilled water as negative control. The plates were incubated at 37°C for 18-24 hours. The diameter of the inhibition zone (mm) was measured.

RESULTS AND DISCUSSION

Collection and preparation of plant materials

Healthy fresh leaves of *Mangifera indica* and *Costus igneus* are collected from the nearby areas of Coimbatore district. The leaves are rinsed with distilled water and dried at room temperature under well ventilated shade. The dried leaves are powdered and stored in air-tight container for further analysis.

Extraction of plant material

The extract is prepared by adding 150 ml of hexane, ethyl acetate and ethanol to 50 g of powdered leaves. After 24 hours, the solvent is allowed to evaporate at room temperature to obtain the hexane, ethyl acetate and ethanol extracts.

Qualitative analysis

Phytochemicals are the potent bioactive components that provide the therapeutic effect in medicinal plants (Doss *et al*., 2009). The results of phytochemical screening of hexane, ethyl acetate and ethanol extracts of *Mangifera indica* are presented in the Table 1.

Table 1. Phytochemical screening of hexane, ethyl acetate and ethanol extracts of *Mangifera indica*

S.No	Phytochemical Tests	Results		
		Hexane Extract	Ethyl Acetate Extract	Ethanol Extract
1	Tannin	+	+	+
2	Saponin	-	-	+
3	Flavonoid	+	+	+
4	Phenol	+	+	+
5	Cardiac glycoside	-	-	-
6	Terpenoid	+	+	+
7	Steroid and Phytosteroid	Steroid +	Steroid +	Steroid +
8	Phlobatannin	-	-	-
9	Alkaloid	+	+	+
10	Carbohydrate	+	+	+

+ = Present - = Absent

Preliminary phytochemical screening of the extracts reveal the presence of various bioactive components like alkaloid, flavonoid, steroid, phenol, terpenoid, carbohydrate and tannin in hexane, ethyl acetate and ethanol extracts. Phlobatannin and Cardiac glycoside are absent in all the extracts. Saponin is present only in ethanol extract. The above results are similar to the study of (Somkuwar and Kamble, 2013) in which the presence of the alkaloids, carbohydrates, tannins, flavonoids were reported in the extracts of *Mangifera indica.* The results of phytochemical screening for hexane, ethyl acetate and ethanol extracts of *Costus igneus* are presented in the Table 2.

Table 2. Phytochemical screening of hexane, ethyl acetate and ethanol extracts of *Costus igneus*

S.No	Phytochemical Tests	Results		
		Hexane Extract	Ethyl Acetate Extract	Ethanol Extract
1	Tannin	+	+	+
2	Saponin	-	+	+
3	Flavonoid	+	+	+
4	Phenol	-	-	+
5	Cardiac glycoside	-	+	+
6	Terpenoid	-	-	+
7	Steroid and Phytosteroid	Steroid +	Steroid +	Steroid +
8	Phlobatannin	+	+	+
9	Alkaloid	+	+	+
10	Carbohydrate	+	+	+

+ = Present - = Absent

Preliminary phytochemical screening of the extracts reveal the presence of various bioactive compounds like flavonoid, steroid, phlobatannin, alkaloid, carbohydrate and tannin in all the three extracts. Cardiac glycoside and Saponin are absent only in the hexane extract. Phenol and terpenoid are present in ethanol extract only. The above results are similar to the study of **(**Pazhanichamy, 2012) in which the presence of the tannin, flavonoid, phlobatannin, terpenoid, saponin, steroid, cardiac glycoside were reported in the leaf extracts of *Costus igneus.* Terpenoid is attributed for analgesic and anti-inflammatory activities and flavonoids had been reported to possess many useful properties, including anti-inflammatory, estrogenic, enzyme inhibition, antimicrobial, antiallergic, antioxidant properties (Harborne and Williams, 2000). Tannins isolated

from plant species *Solanum trilobatum* Linn exhibited antibacterial activities (Doss *et al*., 2009).

Quantitative analysis

Total Phenolic content

Phenolic compounds are among the most important plant components as they possess a variety of biological activities including antioxidant activity, therefore it is quite important to evaluate the total phenolic content in tested extracts (Elzaawely and Tawata, 2010).

Total Phenolic Content in hexane, ethyl acetate and ethanol extracts of *Mangifera indica*

Total Phenolic Content in hexane, ethyl acetate and ethanol extracts of *Mangifera indica* are presented in the Table 3.

Table 3. Total Phenolic Content in hexane, ethyl acetate and ethanol extracts of *Mangifera indica*

Total Phenolic Content		
Concentration (µg/ml)	Name of the extract	Amount of Phenol*
200	Hexane	23.10 ± 1.24
600		54.07 ± 0.79
1000		69.72 ± 1.50
200	Ethyl acetate	33.93 ± 0.97
600		55.05 ± 1.76
1000		74.06 ± 0.91
200	Ethanol	46.74 ± 0.34
600		58.85 ± 1.35
1000		84.14 ± 1.76

*Amount of Phenol is expressed as mg of catechol per gram of extract ** (Values are expressed as mean ± SD)

The ethanol extract has high amount of total phenolics (84.14 mg of catechol per gram of extract) at 1000µg/ml concentration than that of hexane and ethyl acetate extracts (Table 3). This is in accordance with the study of (Elzaawely and Tawata, 2010) in which the ethanol extract of *Mangifera indica* had high phenolic content.

Total Phenolic Content in hexane, ethyl acetate and ethanol extracts of *Costus igneus*

Total Phenolic Content in hexane, ethyl acetate and ethanol extracts of *Costus igneus* is presented in the Table 4.

Table 4. Total Phenolic Content of in hexane, ethyl acetate and ethanol extracts of *Costus igneus*

Total Phenolic Content		
Concentration (µg/ml)	Name of the extract	Amount of Phenol*
200	Hexane	32.21 ± 1.34
600		55.05 ± 1.65
1000		71.99 ± 1.76
200	Ethyl acetate	40.59 ± 0.57
600		58.95 ± 0.65
1000		75.88 ± 1.24
200	Ethanol	51.40 ± 1.98
600		61.59 ± 1.43
1000		88.97 ± 1.78

*Amount of Phenol is expressed as mg of catechol per gram of extract ** (Values are expressed as mean ± SD)

The ethanol extract contain a higher amount of total phenolics (88.97 mg of catechol per gram of extract) at 1000µg/ml concentration than that of hexane and ethyl acetate extracts (Table 4). This is in accordance with the study of (Aruna, 2014) who reported that the ethanol extracts *Costus igneus* leaf had high amount of phenolics.

Total Phenolic Content in ethanol extracts of *Mangifera indica* and *Costus igneus*

Total Phenolic Content in ethanol extracts of *Mangifera indica* and *Costus igneus are* presented in the Figure 1.

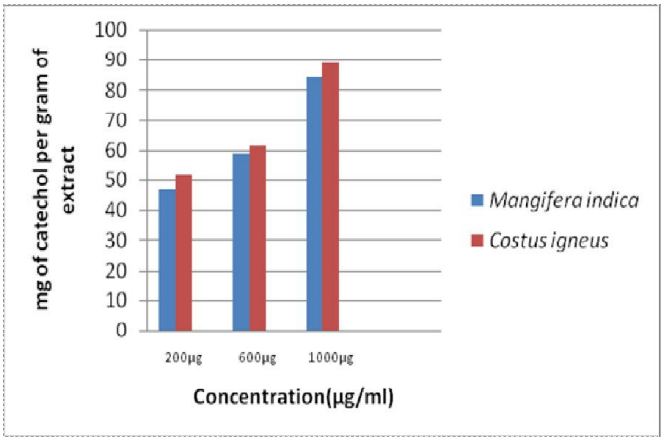


Figure 1. Total Phenolic Content in ethanol extracts of *Mangifera indica* and *Costus igneus*

Among the ethanol extracts of *Mangifera indica* and *Costus igneus* (Figure 1), the amount of total phenolics is found to be high in ethanol extract of *Costus igneus* (88.97 mg of catechol per gram of extract at 1000µg/ml concentration).

***Invitro* antioxidant activity**

DPPH free radical scavenging assay

Scavenging of the stable radical DPPH is considered a valid and easy assay to evaluate scavenging activity of anti oxidants (Nanjo *et al.,* 1996). It is quite important to evaluate the DPPH scavenging activity of plant extracts.

DPPH Scavenging activity of hexane, ethyl acetate and ethanol extracts of *Mangifera indica*

DPPH Scavenging activity of hexane, ethyl acetate and ethanol extracts of *Mangifera indica* are presented in the Table 5.

DPPH scavenging activity of ethanol extract of *Mangifera indica* appeared to be maximum with inhibition of 66% at 1000µg/ml concentrations of plant extract (Table 5). These activities are lesser than ascorbic acid. These results are in accordance to the work of (Kaur *et al.,* 2015) who stated that the ethanol extracts of *Mangifera indica* had high DPPH scavenging activity. The more rapidly the absorbance decreases the more potent is the antioxidant activity of the extract.

Table 5. DPPH Scavenging activity of hexane, ethyl acetate and ethanol extracts of *Mangifera indica*

Concentration (µg/ml)	Name of the extract	Control	DPPH Scavenging Activity	
			% of Inhibition	
			% Inhibition of Sample	% Inhibition of Ascorbic acid*
200	Hexane	1.2339	21.36 ± 0.76	57.04 ± 1.22
600		1.2339	31.91 ± 0.45	74.87 ± 1.42
1000		1.2339	36.59 ± 0.67	89.46 ± 1.31
200		1.2339	27.93 ± 0.56	57.04 ± 1.29
600	Ethyl acetate	1.2339	34.16 ± 0.97	74.87 ± 1.56
1000		1.2339	43.19 ± 1.12	89.46 ± 1.62
200	Ethanol	1.2339	43.19 ± 1.07	57.04 ± 1.86
600		1.2339	61.01 ± 0.99	74.87 ± 1.76
1000		1.2339	65.88 ± 0.77	89.46 ± 1.91

*Ascorbic acid = Standard ** (Values are expressed as mean ± SD)

Table 6. DPPH Scavenging activity of hexane, ethyl acetate and ethanol extracts of *Costus igneus*

Concentration (µg/ml)	Name of the extract	Control	DPPH Scavenging Activity	
			% of Inhibition	
			% Inhibition of Sample	% Inhibition of Ascorbic acid*
200	Hexane	1.2339	23.23 ± 0.22	57.04 ± 1.22
600		1.2339	39.36 ± 0.43	74.87 ± 1.42
1000		1.2339	42.14 ± 0.31	89.46 ± 1.31
200	Ethyl acetate	1.2339	45.36 ± 0.41	57.04 ± 1.29
600		1.2339	50.55 ± 0.54	74.87 ± 1.56
1000		1.2339	57.77 ± 0.67	89.46 ± 1.62
200	Ethanol	1.2339	50.55 ± 0.92	57.04 ± 1.86
600		1.2339	65.88 ± 0.94	74.87 ± 1.76
1000		1.2339	83.78 ± 0.89	89.46 ± 1.91

*Ascorbic acid = Standard ** (Values are expressed as mean ± SD)

Table 7. Anti-inflammatory activity of hexane, ethyl acetate and ethanol extracts of *Mangifera indica*

Concentration (µg/ml)	Name of the plant extract	Control	Anti-inflammatory activity	
			% of Inhibition	
			% Inhibition of Sample	% Inhibition of Aspirin*
250	Hexane	0.87	34.48 ± 0.91	49.42 ± 0.98
500		0.87	43.67 ± 0.87	57.47 ± 1.20
750		0.87	60.91 ± 0.56	71.26 ± 1.43
1000		0.87	68.96 ± 0.67	86.20 ± 1.45
250	Ethyl acetate	0.87	42.52 ± 0.43	49.42 ± 1.98
500		0.87	52.87 ± 0.54	57.47 ± 0.45
750		0.87	64.36 ± 0.61	71.26 ± 0.93
1000		0.87	73.56 ± 0.45	86.20 ± 1.21
250	Ethanol	0.87	44.82 ± 0.32	49.42 ± 1.54
500		0.87	54.02 ± 0.39	57.47 ± 1.61
750		0.87	66.66 ± 0.20	71.26 ± 0.93
1000		0.87	79.31 ± 0.94	86.20 ± 0.65

*Aspirin = Standard ** (Values are expressed as mean ± SD)

Table 8. Anti-inflammatory activity of hexane, ethyl acetate and ethanol of *Costus igneus*

Concentration (µg/ml)	Name of the extract	Control	Anti-inflammatory activity	
			% of Inhibition	
			% Inhibition of Sample	% Inhibition of Aspirin*
250	Hexane	0.87	31.03 ± 0.98	49.42 ± 0.98
500		0.87	37.93 ± 0.78	57.47 ± 1.20
750		0.87	55.17 ± 0.97	71.26 ± 1.43
1000		0.87	64.36 ± 0.45	86.20 ± 1.45
250	Ethyl acetate	0.87	37.93 ± 1.03	49.42 ± 1.98
500		0.87	48.27 ± 1.21	57.47 ± 0.45
750		0.87	58.62 ± 1.54	71.26 ± 0.93
1000		0.87	66.66 ± 1.09	86.20 ± 1.21
250	Ethanol	0.87	42.52 ± 0.99	49.42 ± 1.54
500		0.87	51.72 ± 0.54	57.47 ± 1.61
750		0.87	64.36 ± 0.35	71.26 ± 0.93
1000		0.87	68.96 ± 0.76	86.20 ± 0.65

*Aspirin = Standard ** (Values are expressed as mean ± SD)

DPPH Scavenging activity of hexane, ethyl acetate and ethanol extracts of *Costus igneus*

DPPH Scavenging activity of hexane, ethyl acetate and ethanol extracts of *Costus igneus* are presented in the Table 6. DPPH scavenging activity of ethanol extract of *Costus igneus* appeared to be maximum with inhibition of 84% at 1000µg/ml concentrations of plant extract (Table 6). These activities are lesser than ascorbic acid. This result is in accordance to the work of (Jayasri *et al.,* 2009), who stated that the ethanol extracts of *Costus igneus* had high DPPH scavenging activity. The more rapidly the absorbance decreases the more potent is the antioxidant activity of the extract.

DPPH Scavenging activity of ethanol extracts of *Mangifera indica* and *Costus igneus*

DPPH Scavenging activity of ethanol extracts of *Mangifera indica* and *Costus igneus* are presented in the Figure 2.

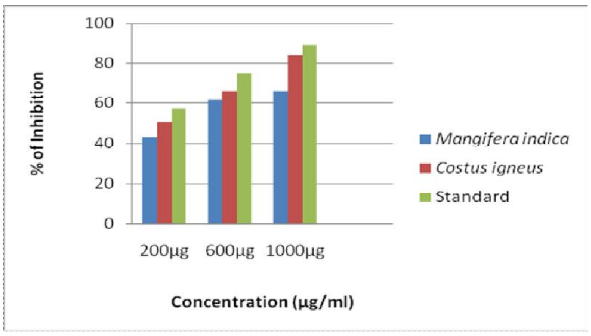


Figure 2. DPPH Scavenging activity of ethanol extracts of *Mangifera indica* and *Costus igneus*

Among the ethanol extracts of *Mangifera indica* and *Costus igneus* (Figure 2), the ethanol extract of *Costus igneus* is found to have more *in vitro* antioxidant potential with inhibition of 84% at 1000 µg/ml concentrations of plant extract.

***Invitro* anti-inflammatory activity**

Inhibition of albumin denaturation

Protein denaturation is a process in which proteins lose their tertiary structure and secondary structure by application of external stress or compound, such as strong acid or base, a concentrated inorganic salt, an organic solvent or heat. Most biological proteins lose their biological function when denatured (Shinde *et al.,* 1999). Denaturation of proteins is a well documented cause of inflammation. Anti-inflammatory drugs act by inhibiting the denaturation of protein. Phenylbutazone, salicylic acid, flufenamic acid (anti-inflammatory drugs) etc, have shown dose dependent ability to inhibit heat induced protein (albumin) denaturation (Mizushima and Kobayashi, 1968). Therefore it is necessary to evaluate the effect of the plant extracts in inhibiting heat induced protein.

Anti-inflammatory activity of hexane, ethyl acetate and ethanol extracts of *Mangifera indica*

The anti-inflammatory activity of hexane, ethyl acetate and ethanol extracts of *Mangifera indica* are presented in the Table 7.

The anti-inflammatory activity of hexane, ethyl acetate and ethanol extracts of *Mangifera indica* are presented in the Figure 3.

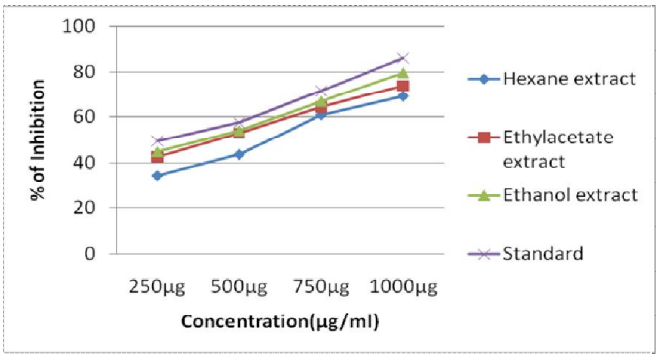


Figure 3. Anti-inflammatory activity of hexane, ethyl acetate and ethanol extracts of *Mangifera indica*

The *in vitro* anti-inflammatory activity of *Mangifera indica* hexane, ethyl acetate and ethanol extracts are represented in the Figure 3. Ethanol extract of *Mangifera indica* has high inhibitory activity on albumin denaturation than hexane and ethyl acetate extracts. The percentage inhibition of ethanol extract of *Mangifera indica* was found to be 79% at 1000 µg/ml concentrations of plant extract. It is significant when compared to the standard aspirin used which possess 86% inhibition of albumin denaturation at 1000 µg/ml concentration. There is a dose dependent increase in the percentage of inhibition. This is in accordance to the work of (Islam *et al.,* 2010) who reported that anti-inflammatory activity was found to be high in ethanol extract of *Mangifera indica* leaves.

Anti-inflammatory activity of hexane, ethyl acetate and ethanol of *Costus igneus*

The anti-inflammatory activity of hexane, ethyl acetate and ethanol extracts of *Costus igneus* are presented in the Table 8.

The anti-inflammatory activity of hexane, ethyl acetate and ethanol extracts of *Costus igneus* are presented in the Figure 4.

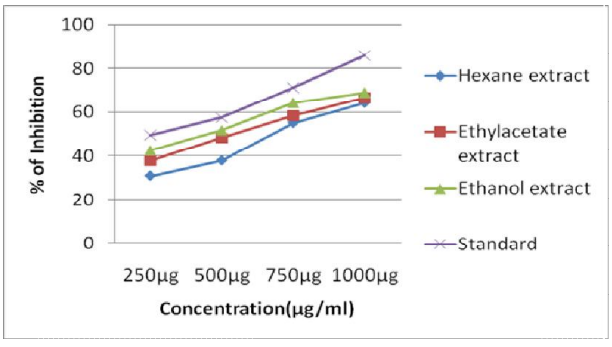


Figure 4. Anti-inflammatory activity of hexane, ethyl acetate and ethanol extracts of *Costus igneus*

The *in vitro* anti-inflammatory activity of *Costus igneus* hexane, ethyl acetate and ethanol extracts are represented in Figure 4. Ethanol extract of *Costus igneus* shows high

inhibitory activity on albumin denaturation than hexane and ethyl acetate extracts. The percentage inhibition of ethanol extract of *Costus igneus* is found to be 69 % at 1000 µg/ml concentrations of plant extract. It is significant when compared to the standard aspirin used which possess 86% inhibition of albumin denaturation at 1000 µg/ml concentration. There is a dose dependent increase in the percentage of inhibition. This is in accordance to the work of (Krishnan *et al.,* 2014) who reported that the *Costus igneus* possess anti-inflammatory activity and it was due to the terpenoid present.

Anti-inflammatory activity of ethanol extracts of *Mangifera indica* and *Costus igneus*

The anti-inflammatory activity of ethanol extracts of *Mangifera indica* and *Costus igneus* are presented in the Figure 5.

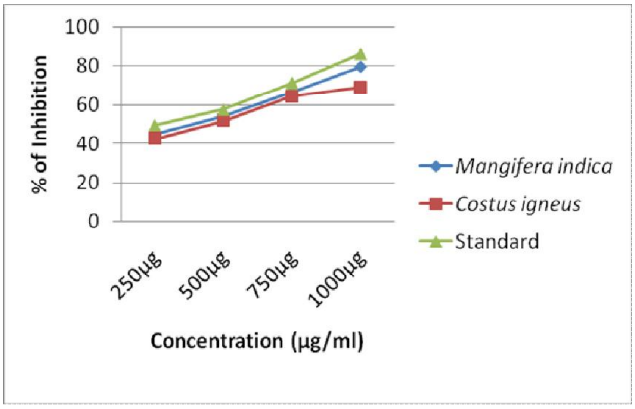


Figure 5. Anti-inflammatory activity of ethanol extracts of *Mangifera indica* and *Costus igneus*

Among the ethanol extracts of *Mangifera indica* and *Costus igneus* (Figure 5), the ethanol extract of *Mangifera indica* shows maximum inhibitory activity on albumin denaturation with 79% inhibition at 1000 µg/ml concentrations of plant extracts.

***Invitro* antibacterial activity**

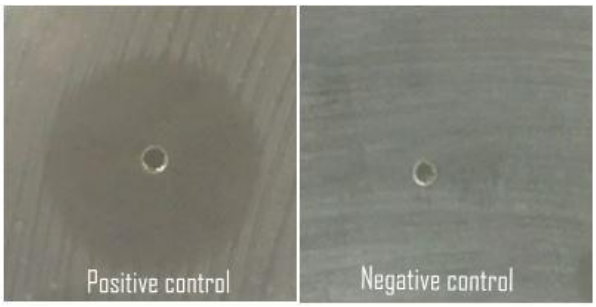
Agar well diffusion method

Plants which are rich in a wide variety of secondary metabolites such as tannins, alkaloids and flavonoid have been found *in vitro* to have antibacterial properties (Lewis and Ausubel, 2006). Therefore it is necessary to evaluate the antibacterial effect of the plant extracts.

Antibacterial activity of hexane, ethyl acetate and ethanol extracts and *Mangifera indica* and *Costus igneus* against *Escherichia coli*

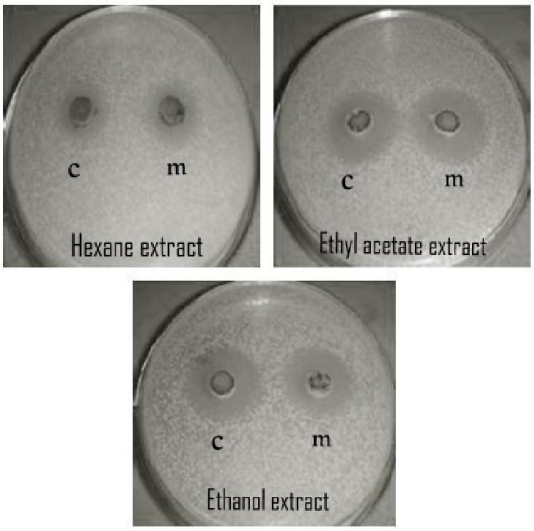
The positive and negative control against *Escherichia coli* are shown in the Figure 6.

The antibacterial activity of hexane, ethyl acetate and ethanol extracts and *Mangifera indica* and *Costus igneus* against *Escherichia coli* are shown in the Figure 7.



***Positive control = Gentamycin**
***Negative control = Distilled water**

Figure 6. Positive and negative control against *Escherichia coli*



c = *Costus igneus
m = *Mangifera indica

Figure 7. Antibacterial activity of hexane, ethyl acetate and ethanol extracts and *Mangifera indica* and *Costus igneus* against *Escherichia coli*

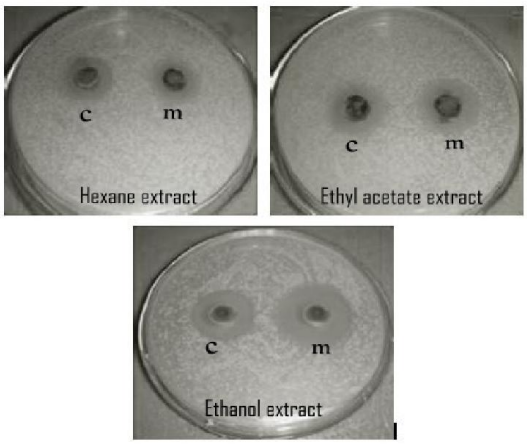
Antibacterial activity of hexane, ethyl acetate and ethanol extracts and *Mangifera indica* and *Costus igneus* against *Staphylococcus aureus*

The positive and negative control against *Staphylococcus aureus* are shown in the Figure 8.

***Positive control = Gentamycin**
***Negative control = Distilled water**

Figure 8. Positive and negative control against *Staphylococcus aureus*

The antibacterial activity of hexane, ethyl acetate and ethanol extracts and *Mangifera indica* and *Costus igneus* against *Staphylococcus aureus* are shown in the Figure 9.



c = *Costus igneus* *m = *Mangifera indica

Figure 9. Antibacterial activity of hexane, ethyl acetate and ethanol extracts and *Mangifera indica* and *Costus igneus* against *Staphylococcus aureus*

The antibacterial activity of *Mangifera indica* is shown in the Table 9.

Table 9. Antibacterial activity of *Mangifera indica*

Bacteria	Zone of inhibition(mm)			
	Mangifera indica			Control
	Hexane extract	Ethyl acetate extract	Ethanol extract	Gentamycin
Escherichia coli	10	13	17	21
Staphylococcus aureus	11	12.5	14	17

Antibacterial activity of *Mangifera indica* leaves extract is found good against gram positive and gram negative bacteria. More specifically hexane, ethyl acetate and ethanol extracts of *Mangifera indica* leaves extracts shows 11.0 mm, 12.5 mm and 14 mm in diameter of zone of inhibition against *Staphylococcus aureus* which is a gram positive bacterium. Table 9 shows the zone of inhibition as 10 mm, 13 mm and 17 mm for hexane, ethyl acetate and ethanol extracts of *Mangifera indica* leaves extracts respectively against *Escherichia coli* which is a gram negative bacterium. (Doughari *et al*., 2008) reported the antibacterial property of *Mangifera indica* leaf extracts against gram positive and gram negative bacteria.

The antibacterial activity of *Costus igneus* is shown in the Table 10.

Table 10. Antibacterial activity of *Costus igneus*

Bacteria	Zone of inhibition(mm)			
	Costus igneus			Control
	Hexane extract	Ethyl acetate extract	Ethanol extract	Gentamycin
Escherichia coli	9.5	12	14	21
Staphylococcus aureus	9	10.5	11	17

Table 10 shows the zone of inhibition of hexane, ethyl acetate and ethanol extracts of *Costus igneus* leaves extracts as 9mm, 10.5mm and 11mm respectively against *Staphylococcus aureus* which is a gram positive bacterium. The zone of inhibition is 9.5 mm, 12 mm and 14 mm for hexane, ethyl acetate and ethanol extracts of *Costus igneus* leaves extracts respectively against *Escherichia coli* which is a gram negative bacterium. Antibacterial activity of *Costus igneus* leaves extract is found moderate against gram positive and gram negative bacteria. (Gothandam *et al*., 2010) reported that *Costus igneus* showed maximum anti-bacterial activity against *gram-positive* and gram-negative bacteria. By comparing the zone of inhibition of ethanol extract of *Mangifera indica* (Table 9) and ethanol extract of *Costus igneus* (Table 10), it is found that the ethanol extract of *Mangifera indica* shows maximum zone of inhibition against *Escherichia coli* and *Staphylococcus aureus* with a diameter of 17mm and 14mm respectively.

Conclusion

From the above findings it can be concluded that antioxidant, anti-inflammatory and antibacterial activities are due to the presence of phytoconstituents in the plants. The ethanolic extract of *Mangifera indica* has maximum anti-inflammatory and antibacterial activity. Since the leaves of *Mangifera indica* are more efficient than *Costus igneus,* it can be used in treating inflammation and associated bacterial infections. The current study therefore provides the scientific basis for the traditional application as ethno medicine.

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