Evaluation of *In Vitro* Anti-inflammatory and Antibacterial Activities of Leaves, Roots and Combination of Leaves and Roots Extracts of Plant *Magnolia figo*

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Abstract

Presently there is a rising demand for novel and potent anti-inflammatory drugs of plant origin owing to the lesser amount of side effects compared to Non-Steroidal anti-inflammatory Drugs. On the other hand, microbial resistance to conventional antimicrobial agents has increased during the last three decades. This study aims to overcome these challenges by investigating an herbal plant application. It was designed to evaluate in vitro anti-inflammatory and antibacterial activity of methanolic extracts of leaves, roots and combination of both leaves and roots extracts of *Magnolia figo*; a native Chinese medicinal plant. In vitro anti-inflammatory property was determined using heat-induced protein (egg albumin) denaturation test using diclofenac sodium as the positive control. Antibacterial activity of the methanolic extract was evaluated against *Escherichia coli* (ATCC® 25922TM) and *Staphylococcus aureus* (ATCC® 25923TM) using the cylinder plate method. As a result, considerable dose-dependent anti-inflammatory activities were detected in the combination extract (IC50 =1.819 µg/mL) when compared to the positive control (diclofenac sodium) (IC50 =4.337 µg/mL). Among the extracts, the highest effect against *E. coli* was exhibited by the combination extract (diameter of inhibitory zone =15.30 mm). None of the extracts showed a positive antibacterial effect against *S. aureus*. Phytochemical investigations of extracts indicated the presence of alkaloids, flavonoids, phenols, tannins, saponins, terpenoids, glycosides and steroids. This was concluded a significant result elicited by the combination of plant parts confirm that *M. figo* is a medicinal plant that can be used to develop novel anti-inflammatory and antibacterial agents.

Keywords: Anti-inflammatory, Antibacterial, Phytochemical analysis, *Magnolia figo*, *Escherichia coli*, *Staphylococcus aureus*

Introduction

Traditional herbal remedies are more acceptable in most ethnic societies as compared to allopathic medicines because they are considered to be the safest approach to treat diseases with the least side effects on human health. Nature has provided abundant plant wealth for all living creatures which create a medicinal virtue. The total number of the 252 drugs in the WHO essential medicine list,11% of medicines are exclusive of plant origin[1].The bioactive compounds of medicinal plants are used as antidiabetic, chemotherapeutic, anti-inflammatory, anti-arthritic agents where there is no satisfactory cure in modern medicines[2].

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Hence, the present study aims to determine the anti-inflammatory and antibacterial activity of selected plant parts of *Magnolia figo*.

Inflammation is the bodily response to an injurious stimulus evoked by a wide variety of noxious agents, for example, physical trauma, noxious chemicals or microbial agents[3]. Studies have shown that single or multiple bacterial origins can be the cause for inflammation where the microbial invasion stimulates acute inflammation in to a chronic inflammation[4].The commonly used drugs for the management of inflammatory conditions are non-steroidal anti-inflammatory drugs (NSAIDs) which have several side effects especially gastric irritations leading to the formation of gastric ulcers, cardiovascular risks and hormonal imbalance. Instead of side effects causing NSAIDs, the rich wealth of the plant kingdom has been used to represent a novel source of compounds with anti-inflammatory activities[3]. The denaturation of protein is one of the causes of inflammation. In certain rheumatic diseases, the production of auto antigens maybe due to the denaturation of proteins. Anti-inflammatory drugs are known to inhibit the denaturation of proteins. Therefore, agents that can prevent denaturation could be used for the development of anti-inflammatory drugs and anti-arthritic drugs[5].

The emergence of new infectious diseases, the resurgence of several infections and the increase in bacterial resistance have created the necessity for studies directed towards the development of new antimicrobial agents. Most bacteria, fungi and viruses have a lesser ability to develop resistance against the botanicals. Alteration of target sites, active efflux of drugs and enzymatic degradations are the strategies employed by the pathogenic bacteria to develop intrinsic resistance to antibiotics. Secondary metabolites of plants such as alkaloids, tannins, polyphenols etc. could act as potentials for antimicrobials and resistance modifiers. Plant extracts can bind with protein domains leading to modification or inhibition of protein-protein interactions[6].

The Magnoliaceae is a family of about 220 species of deciduous or ever green trees and shrubs native to Asia and America with large showy flowers. Based on the pharmacological actions Magnoliaceae plants report three categories of features: biochemical (37.5%), cardiovascular (20.8%) and CNS pharmacology (25.0%)[7].

Magnolia figo plant which belongs to the Magnolia (Family Magnoliaceae) genus are arboreous plants growing in the temperate zone. Though it grows in China, now it is cultivated both in the hill-country and mid-country in Sri Lanka. It is commonly called as banana shrub, because of the heavy, sweet banana scent of its purple flower. The seeds and flowers are used for making preparations for strengthening sexual virility. The bark is a stimulant, expectorant, astringent and the root is a purgative. There is no scientific evidence available on *M. figo* leaves and roots corresponding to their in vitro evaluation of anti-inflammatory activity and antibacterial activity. The leaves of this shrub contain alkaloids, magnolamine, magnoline and tetrandrine[8].

This study is designed to evaluate the anti-inflammatory and antibacterial action of *M. figo* plant parts including leaves and roots as the first approach of developing newer drugs that can be used as anti-inflammatory drugs with fewer side effects and antibacterial drugs with less microbial resistance. Furthermore, phytochemical screening of *M. figo* plant parts and the comparison of their anti-inflammatory and antibacterial activities will be a remarkable approach for the development of a novel therapeutic drug.

Materials and Methods

Study Location

The study was carried out in the Chemistry Laboratory and Research Laboratory, General Sir John Kotelawala Defense University, Rathmalana and Pharmacy Skills Laboratory, Faculty of Allied Health Sciences in General Sir John Kotelawala Defense University, Werahera, Sri Lanka.

Plant collection and authentication

About 800g of each matured, fully expanded leaves and roots of *M. figo* were collected from Peradeniya, Kandy in fresh condition at day time after studying

the morphological and organoleptic properties carefully. The plant voucher specimens (NH/ BOT/4/2019-51(II)) were deposited according to the herbarium rules and regulations and authenticated by National Herbarium, Royal Botanical Garden, Peradeniya, Sri Lanka. The collected plant materials were thoroughly cleaned using running tap water and air-dried until a constant weight was obtained.

Preparation of crude plant material extracts

Powdered samples were used for the extraction procedure. The methanolic extract was obtained by cold maceration. The alcohol was distilled off from the filtrate under reduced pressure to obtain a dry residue. This extract was stored in a desiccator at a low temperature until the actual experiment was done[5].

Heat-induced protein denaturation test

A concentration series of plant extracts were compared with the same concentration of the positive control for the anti-inflammatory activity. Diclofenac sodium was used as the standard anti-inflammatory drug. A similar volume of double distilled water was used as the negative control. Egg albumin was obtained by centrifugation of diluted fresh chicken egg white and samples were added for the preparation of the reaction mixture. The pH of the egg albumin was adjusted by adding freshly prepared phosphate buffer saline (PBS, pH=6.4). Albumin (270µl) and 30µl of different concentrations of plant samples were inserted into each well from rows of A to C and columns of 96 well flat bottom microtiter plate and triplicated the reaction mixture. Albumin (270µl) and 30µl of negative control samples were added into each well in column D. Albumin (270µl) and 30µl of positive control samples were inserted into each well in rows of E and F and columns. Albumin (300µl) was inserted into each well in rows of G and H columns. Mixtures were incubated at 37°C (37±2°C) for 10 to 15 minutes. The denaturation process was induced by increasing the temperature gradually up to 57° Cand kept for 5 minutes. Samples were allowed to cool down to room temperature at 30°C. After cooling down, the absorbance was measured at 660nm using an ELISA plate reader. The percentage inhibition of protein denaturation for each sample was calculated by using the absorption readings according to the equation.

Percentage of inhibition = 100*[Vt/Vc-1]

Where Vt = absorbance of the test sample and Vc = absorbance of control[5]

The dose-response curve of each sample was compared with each other and the positive control. The 50% inhibition (IC50) of extract drug concentration was determined by plotting percentage inhibition with respect to control against treatment concentration.

Statistical analysis

The results are presented as mean,±SEM (Standard Error of Mean) and ±SD (Standard Deviation) was calculated by descriptive statistical analysis and correlation analysis using Statistical Package for Social Sciences (SPSS) version 25. A significant level was set up at $p<0.05$. The concentration dependencies and IC50 value (half maximal inhibitory concentration) were calculated by affecting nonlinear regression using the Graph pad Prism 9 statistical software package.

Cylinder plate antibiotic assay method

Stock cultures of *Escherichia coli* (ATCC 25922) and *Staphylococcus aureus* (ATCC 25923) were obtained as common wound infecting bacteria. A serial dilution series was prepared by re-dissolving crude extract in Dimethyl sulfoxide (DMSO) starting from 1500µg/mL filtrate up to 250µg/mL. In this study, gentamycin (50 µg/ mL) was used as the positive control[9]. It was prepared by using a commercially available gentamycin (40 mg/mL) IV injection vial. Sub cultures were prepared using bacteria (*Escherichia coli* and *Staphylococcus aureus*) in nutrient agar slants and incubated at 37°C for 24 hours to obtain pure bacterial colonies to prepare the bacterial broths. Two to three bacterial colonies were obtained from subcultures using a sterile inoculating loop and dissolved in 5mL of 0.9% sterile normal

saline under aseptic conditions. Then the bacterial suspension was compared with previously prepared McFarland turbidity standards. Turbidity of bacterial suspension was adjusted to get equivalent with 0.5 McFarland standard. Turbidity of 0.5 McFarland standard was equivalent to that of a bacterial suspension of 1.5 × 108 CFU/mL.

Two bacteria suspensions (1 mL) were added to the 45mL nutrient agar containing flasks. Bacteria suspension was added into agar at 40°C of temperature by using a sterile 1mL pipette under sterile conditions. Cylinders were placed according to a template positioned under the Petri plate which made uniform gaps between cylinders. All the procedures were carried under strict aseptic conditions[10]. Gentamycin (50 µg/ mL) was used as the positive control and DMSO was used as the negative control. Each step of the experiment was repeated as triplicates. Plates were kept at room temperature (25°C – 30°C) for 2 hours to allow diffusion of extracts into the agar. All the plates were incubated at 37°C in an incubator for 24 hours[11]. After incubation, the diameter of the inhibition zones was measured using a vernier caliper.

Phytochemical screening of the M. figo roots and combination extracts

Phytochemical screening of roots and a combination of roots and leaves were done using previous study methods. Accordingly, Flavonoids (Alkaline Reagent test)[12], Carbohydrates (Molisch Reagent test)[13], Tannins (Braymer's test)[14], Saponins (Froth test)[12], Alkaloids (Wagner's test)[15], Glycosides (Keller-Kiliani Test)[14], Phenols (Ellagic acid Test)[14], Terpenoids (Salkowski test)[13], Steroids (Liberman Burchard test)[13], amino acids and proteins (Ninhydrin Test)[15] levels in the selected plant parts of *M. figo* were detected.

Results

Anti-inflammatory effect of methanolic leaves, roots and combination (leaves and roots) extracts of Magnolia figo

The combination extract of leaves and roots showed the highest percentage of inhibition when compared to the other two extracts. The leaf extract showed the next highest percentage inhibition at the 1000 µg/mL concentration. Concentration at 31.25 µg/mL also, the second-highest percentage inhibition was shown by the leaf extract. At the concentration of 0.5 µg/mL, the extract of the root has shown the second-highest percentage inhibition among the three types of extracts (Table 1).

Figure (1) shows that, with the increasing log concentrations of *M. figo* leaves extract, percentage inhibitions were also increasing.

Combination extract and leaves extract showed higher potencies compared to diclofenac sodium. Root extract showed lower potency compared to diclofenac sodium (Table 2).

Antibacterial effect of methanolic leaves, roots and combination (leaves and roots) extracts of Magnolia figo against Escherichia coli

According to the results (Table 3), the highest antibacterial effect against *E. Coli* was expressed by methanolic roots and leaves combination extract, with a concentration of 1500µg/mL and the inhibitory zone was 15.30mm. The lowest antibacterial effect against *E. Coli* was expressed by roots extract while leaves extract showed a moderate effect. Mean inhibition zones of extracts with a concentration of 1500µg/mL of leaves and roots extracts were 15.10mm and 14.45mm respectively.

All the extracts of *M. Figo* showed the highest antibacterial effect when the concentration of the sample was 1500µg/mL. All the extracts exhibited an antibacterial effect against *E. coli* in all the five concentrations (1500µg/mL, 1000µg/mL, 750µg/mL, 500µg/mL, 250µg/mL) tested. None of the *M. figo* plant extracts exhibited a positive antibacterial effect against *S. Aureus* as none of the extracts showed an inhibitory zone compared to the negative control. All values were around 10mm (Table 4).

According to the data obtained by EC50 values and dose-response curve, methanolic combination extract of *M. Figo* exhibited the highest efficacy and highest potency and at the same time the methanolic roots extract and leaves extract exhibited lower potencies than combination extract (Figure 2).

According to the above studies (Table 5), it was found that $p<0.05$ which indicates that the data is significant. Further, all R^2 values were found to be nearly 1 and it shows that there is a positive correlation between the zone of inhibition and concentrations of all three plant extracts.

Phytochemical study results indicated the presence of alkaloids, flavonoids, carbohydrates, phenols, tannins, saponins, protein and amino acid, terpenoids, glycosides and steroids in the extracts of the combination of leaves and roots of *M. Fig o*plant whereas roots confirm the absence of saponins, glycosides and terpenoids (Table 6).

Discussion

Studies conducted for the evaluation of sunscreen activity and antioxidant activity of methanolic leaf extract of *M. figo*; phytochemical screening indicate that leaf extracts are rich in alkaloids, phenols, tannins, terpenoids, flavonoids, steroidal glycosides and saponins.The results concluded that leaf extract of *M. Figo* possesses marked antioxidant activity and sunscreen activity which exhibit its potential use for the prevention of oxidative stress[16].

According to the literature reviews, the antibacterial activity and anti-inflammatory activity of methanolic leaf and roots extracts of *M. Figo* were not evaluated in Sri Lanka in the past. The study was conducted using the methanolic extracts of the plant parts of *M. Figo* to overcome the problems that occurred in conventional medicines used in inflammations and bacterial infections. The anti-inflammatory study was carried out by using the egg albumin denaturation test. As the egg albumin was not denatured, it was confirmed that those plant extracts contain phytoconstituents which have anti-inflammatory properties. The absorbance of denatured samples was analyzed by using an ELISA plate reader. The absorbance of the plant sample was compared with the diclofenac Na drug sample and inhibition of albumin denaturation was identified. Variations in hydrogen, hydrophobic, electrostatic and disulphide bonding would have taken place due to the mechanisms of denaturation[17]. PBS was used to maintain pathological pH (pH 6.2-6.5).

In this investigation, the methanolic combination extract of *M. figo* exhibited greater inhibition of protein denaturation than the anti-denaturation effect of the standard drug diclofenac sodium. It is noticeable that except for roots extract, all other plant extracts showed a higher potency compared to diclofenac sodium. Diclofenac sodium exhibits an IC50 value of 4.337 µg/mL and a high R2 (0.9220) indicating a strong positive relationship with the inhibitory percentage and log concentrations. On the other hand, the combination extract curve showed a similar pattern to diclofenac sodium. On the whole, results show that combination extract overcomes the action of the reference drug. It showed a higher or synergistic anti-denaturation effect compared to the other two extracts of leaves and roots. The methanolic leaves, roots and combination extracts showed marked dose - dependent anti-denaturation effects indicating potent anti-inflammatory activity with an IC_{50} value of 4.132µg/mL, 6.519µg/mL, and 1.819µg/mL respectively (IC_{50} value of diclofenac sodium is 4.337µg/mL). There was a strong positive statistically significant ($R^2=0.8307$, $p<0.001$) correlation between concentration and percentage inhibition by leaves extract of *M. figo*. There was a moderate positive statistically significant ($R^2= 0.4422$, $p<0.001$) correlation between concentration and percentage inhibition by roots extract of plant *M. figo*. There was a relatively strong positive statistically significant ($R^2= 0.5684$, $p<0.001$) correlation between concentration and percentage inhibition by combination extract of plant *M. Figo* ($R^2= 0.9220$, $p<0.001$ of diclofenac sodium).

Table 1: Percentage inhibition for extract samples of *M. figo* plant parts and reference drug

Concentrations (µg/mL)	Leaves	Roots	Combination	Reference Drug (Diclofenac Na)
1000	86.365±12.2	61.389±7.5	97.895±25.3	96.128±0.3
500	85.299 ± 7.3	58.977±9.9	93.468±8.6	95.674±3.6
250	61.293 ± 1.3	56.345±6.5	82.418±19.8	89.002±1.0
125	61.151 ± 3.5	66.910±3.1	81.748±15.8	90.377±11.1
62.5	62.225 ± 2.3	41.983±5.7	79.179±12.0	76.992±0.4
31.25	64.140 ± 0.76	47.908±16.6	66.236±10.1	70.808±3.5
15.625	36.679 ± 6.62	43.465±1.1	68.74±16.2	68.732±1.8
7.8125	20.282 ± 0.76	20.887±2.1	54.684±11.7	64.329±0.7
3.9	9.877± 1.32	16.387±0.1	38.86±12.367	41.518±11.2
1.95	6.363 ±5.51	27.125±2.8	25.239±23.3	20.945±0.1
1	0.324 ±0.76	31.956±1.2	21.421±17.2	15.129±6.1

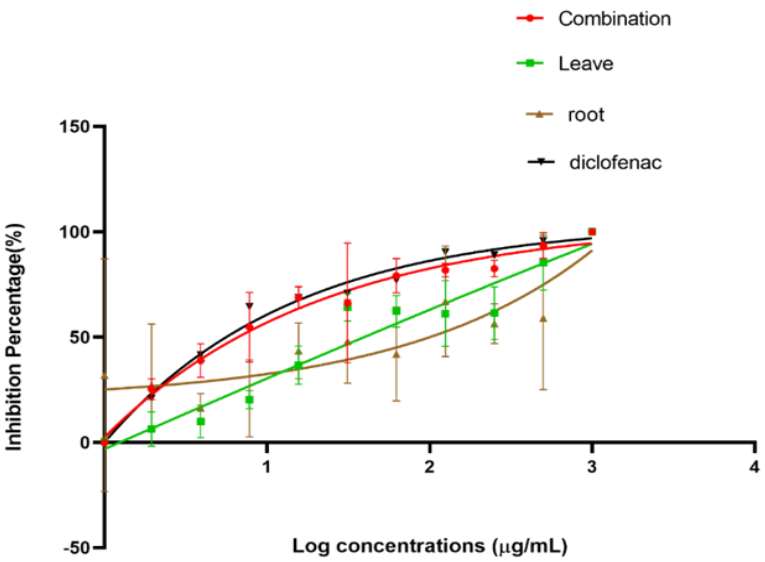

Figure 1: Dose-response curves for anti-inflammatory properties of the *M. figo* plant leaves, roots and combination extracts and reference drug (diclofenac sodium) based on inhibition percentage.

Table 2: Details of the dose-response curves of reference drug and *M. Figo* leaves, roots and combination (leaves, roots)

Tabular results	**Leaves**	**Roots**	**Combination**	**Reference drug (Diclofenac Na)**
IC_{50}(µg/mL)	4.132	6.519	1.819	4.337
R^2	0.8307	0.4422	0.5684	0.9220
P value	<0.0001***	<0.0001***	<0.0001***	<0.0001***

*** P<0.001

Table 3: Antibacterial effect of methanolic leaves, roots and combination extracts of *M. Figo* against *E. Coli.*

Concentrations (µg/mL)	Zone of inhibitions of *Magnolia figo* plant parts		
	Leaves	Roots	Combination
1500	15.10±0.12	14.45±0.35	15.30±0.04
1000	14.14±0.34	14.11±0.13	15.31±0.18
750	13.21±0.20	12.52±0.15	14.51±0.50
500	12.20±0.25	12.63±0.15	13.47±0.17
250	10.86±0.22	11.47±0.21	12.92±0.60
Positive control	30.84±1.19	28.61±2.06	29.78±1.98
Negative control	10.18±0.05	10.21±0.34	10.33±0.30

(Data is expressed as, mean inhibitory diameter ±SEM/ Positive Control-Gentamycin 50 µg/ml/ Negative control - DMSO (Dimethyl Sulfoxide))

Table 4: Antibacterial effect of methanolic leaves, roots and combination extracts of *M. figo* against *S. aureus.*

Concentrations (µg/mL)	Zone of inhibitions of *Magnolia figo* plant parts		
	Leaves	Roots	Combination
1500	10.33 ± 0.3	10.66 ± 0.2	9.96 ± 0.2
1000	10.15 ± 0.3	10.61 ± 0.2	10.22 ± 0.3
750	10.28 ±0.5	10.52 ± 0.2	10.44 ± 0.4
500	10.54 ± 0.1	10.36 ± 0.3	10.34 ± 0.3
250	10.12 ± 0.1	10.51 ± 0.1	10.26 ± 0.2
Positive control	30.84 ± 1.2	30.16 ± 2.0	28.37 ± 2.1
Negative control	10.18 ± 0.1	10.22 ± 0.8	10.33 ± 0.3

(Data is expressed as, mean inhibitory diameter ±SEM/Positive control- Gentamycin 50 µg/ml/Negative control - DMSO (Dimethyl Sulfoxide))

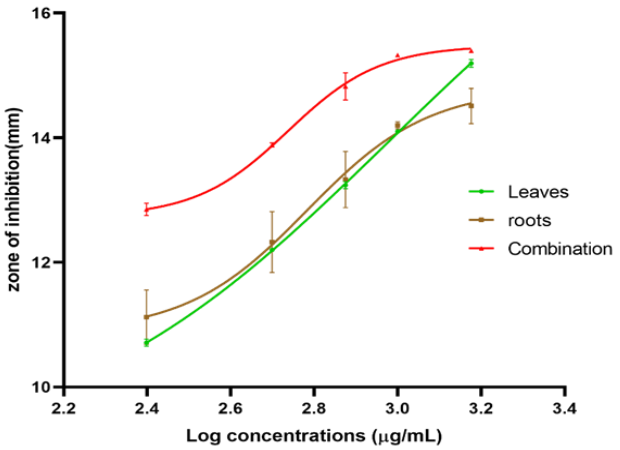

Figure 2: Dose-response curves of methanolic leaves, roots and combination extracts of *M. figo* against *E. Coli*

Table 5: Details of the dose-response curves of plant extracts against *E. Coli.*

Escherichia coli	**Leaves**	**Root**	**Combination**
EC_{50} (µg/mL)	946.5	606.7	536.2
Log EC_{50}	2.976	2.783	2.729
p value	0.0001*	0.0002*	0.0005*
R^2	0.9994	0.9521	0.9986

*p<0.05

Table 6: **Phytochemical analysis of *M. figo* plant.**

Phytochemical	**Test**	**Results**	
		Root	**Combination (leaves+roots)**
Flavonoids	Alkaline Reagent test	+	++
Carbohydrates	Molisch Reagent test	+++	+++
Tannins	Braymer's test	+	+++
Saponins	Froth test	-	++
Alkaloids	Wagner's test	+	+++
Glycosides	Keller-Kiliani test	-	++
Phenols	Ellagic acid test	+	+++
Aminoacids and proteins	Ninhydrin test	+++	+++
Terpenoids	Salkowski test	-	+
Steroids	Liberman Burchard test	++	+

(Mild presence: (+) / Moderate presence: (++) / High presence: (+++)

Thus, methanolic extract of leaves shows a much higher correlation between concentration and percentage inhibition than roots and combination extracts of the plant. From the above results, it is concluded that the methanolic combination extract of *M. Figo* possesses considerable anti-inflammatory activity *invitro* and this is a novel finding. Anti-inflammatory activity is likely to be mediated via the synergistic effect of flavonoids, alkaloids, tannins, saponins, phenols, steroids, glycosides and terpenoids[18].

According to the findings, the presence of higher amounts of phenols, flavonoids, tannins, saponins, terpenoids, steroidal glycosides and alkaloids in combination extract was shown compared to the other two extracts of the plants. As such, it can be assumed that the above secondary metabolites caused the synergistic effect in the combination extract. A wide range of flavonoids with various chemical structures was associated with different anti-inflammatory effects by significantly inhibiting several numbers of inflammatorymediators[19]. Terpenoids also possess significant analgesic and anti-inflammatory

activities. Such activity has been attributed to the ability to inhibit phospholipase A_2 and there by ultimately blocking the metabolism of arachidonic acid. Several alkaloids may also prevent inflammation by blocking the metabolic pathway of arachnoid acid[20].

Polyphenols reduce blood pressure, inflammation and work as oxidative markers and also, they prevent endothelial dysfunction, they are antithrombotic, and they act as vasodilators. They also inhibit the proinflammatory activity of Cyclooxygenase (COX), Lipoxygenase (LOX) and Inducible Nitric Oxide Synthase (iNOS)[21]. Tannins could affect the inflammatory response via free radical scavenging properties and inhibition of iNOS in macrophages, whereas saponins inhibit pain and inflammation via Nitric Oxide (NO) inhibition[18]. Steroidal glycoside called tomatine proves to be effective as an anti-inflammatory agent in humans without exerting the serious side effects observed with both the steroid and non-steroid anti-inflammatory drugs, it may be used in the treatment of chronic inflammatory diseases[22].

Gram-negative *E. coli* (ATCC 25922) andgram-positive *S. aureus* (ATCC 25923) were used to evaluate the antibacterial activity of leaves, roots and combination (leaves and roots) extracts of plant *M. Figo* using cylinder plate method. Gentamycin; the positive control of our study is a broad-spectrum bactericidal agent against many aerobic gram-negative bacteria, but has lower activity against most gram-positive organisms with the exception of staphylococci. The concentration of 50µg/mL was prepared because the gentamycin concentration range for antibiotic sensitivity testing should be within 0.003-64µg/mL. The results of the study show that the methanolic combination extract exhibited the highest zone of inhibition (15.30mm) against gram-negative *E. coli.* Roots extract of *M. Figo* exhibited the lowest inhibition (14.45mm) against gram-negative *E. coli.* Leaves extract exhibited moderate inhibition (15.10mm) against *E. coli*. All three methanolic extracts have no positive inhibition against gram-positive *S. aureus.* (diameter of the well in the agar plate=8mm).

The results revealed that 1500µg/mL concentration of all three extracts had the highest zones of inhibition. Accordingly, concentrations of the extracts have shown a positive correlation with the zone of inhibition against *E. coli* with R^2 values approximately equal to 1. Extracts at the concentration of 1500µg/mL have shown their zone of inhibition approximately between 15.30mm-14.45mm which is much closer numerically. Further, they have shown a statistical significance ($p<0.05$) between their mean zones of inhibition against *E. coli* at a concentration of 1500µg/mL. Yet, they have shown a mean zone of inhibition which is less than the positive control (approximately 30mm). Besides, they have not exhibited an effective antibacterial activity at the concentration of 250µg/mL. None of the concentrations have shown a positive antibacterial response against *S. aureus*. Yet, the positive control has shown a zone of inhibition approximately equal to 30mm. Hence, methanolic extracts of leaves, roots and a combination of *M. Figo* are not effective as an antibacterial agent against *S. Aureus.* Analyzing the data obtained from the dose-response study, the highest EC_{50} value (946.5µg/mL) against *E. coli* is exhibited by methanolic leaves extract whereas the lowest EC_{50} value (536.2µg/mL) was obtained from methanolic combination extract. Methanolic combination extract has shown the highest antibacterial potency and methanolic leaves extract has the lowest antibacterial potency against *E. coli.*

According to these results, methanolic extracts of leaves, roots and the combination of *M. Figo* have shown a positive antibacterial response against gram-negative bacteria *E. coli* (ATCC 25922) and negative antibacterial response against gram-positive bacteria namely *S. aureus* (ATCC 25923). This indicates that leaves, roots and combination of *M. figo* extracts may possess a gram-negative antibacterial spectrum. Thus *M. figo* plant has the potential in the development of a novel antibacterial medicine with no antibiotic drug resistance.

The highest effect of anti-inflammatory activity was shown by the combination extract of *M. Figo* followed by its leaves extract and the root extract and

the highest effect of antibacterial activity was also shown by the combination extract of *M. Figo* followed by its root extract and leaves extract**.** The variable presence of phytoconstituents in different parts of the plant such as roots and leaves must have been the reason for the above observations**.**

M. figo roots extract showed a high presence of carbohydrates, amino acids and proteins with the moderate presence of phytochemicals such as steroids**.** Flavonoids, saponins and glycosides were present in the combination extract as moderately present phytoconstituents**.** On the whole, it can be concluded that the combined extract of *M. Figo* is a richer source of phytoconstituents than the leaves and roots extracts which would rise as a good antibacterial and anti-inflammatory activities**.** Flavonoids and phenols exert their antibacterial activity via interactions with proteins on the bacterial cell wall and thereby disrupting their function and integrity**.** Saponins also have been shown to induce antibacterial actions through a membranolytic action by increasing the permeability of bacterial cell wall[23]**.** Alkaloids are also believed to elicit antimicrobial and trypanocidal activity by inhibition of protein biosynthesis and by interaction with neuroreceptors**.** In the near future, it can be expected that phytochemicals-based drugs will be an object of growing interest for inflammatory and bacterial related diseases**.**

Conclusion

This study showed that methanolic extracts of *M. figo* plant parts (leaves, roots) have marked *in vitro* dose-dependent anti-inflammatory activity and antibacterial activity. The anti-inflammatory activity of methanolic leaves and combination extracts of the plant were more potent than the reference drug. *M. figo* extracts showed marked antibacterial activity against *E. coli*, but not as effective as the reference drug (gentamicin). These anti-inflammatory and antibacterial activities may be mediated through the synergistic effect of the secondary metabolites present in the *M. figo* plant. Further studies are necessary to establish the mechanism of action and ascertain the active constituents responsible for the pharmacological activities of the extracts of *M. figo* plant.

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Conflict of interest

Authors had declared that they have no conflict of interest.

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