Evaluation of Anti-inflammatory and Antibacterial activities of the extracts of leaves, roots and combination of leaves and roots of plant *Magnolia figo.*

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Abstract:-Discovery of novel drugs from medicinal plants is getting popular owing to lesser side effects and to overcome antimicrobial resistance. The methanolic extracts of both leaves and roots of *Magnolia figo* plant by cold maceration were subjected to the evaluation of anti-inflammatory and antibacterial activity. *In vitro* anti-inflammatory property was determined using heat-induced protein (egg albumin) denaturation test compared to diclofenac sodium (positive control). Concentration series of the extracts were analyzed to calculate the percentage inhibition (IC_{50}) of heat-induced protein denaturation. Antibacterial activity of the methanolic extract was determined against *Escherichia coli* (ATCC® 25922™) and *Staphylococcus aureus* (ATCC® 25923™) using the cylinder plate method using gentamycin as the positive control. The size of inhibitory zone was compared with the positive control to determine the antibacterial activity. Lower IC 50 value (1.819 (µg/mL)) was shown in the combination extract of *M. figo* plant compared to the reference drug (4.337 (µg/mL)). It reflects the synergistic effect of the plant parts. The leaves and roots combination extract exhibited dose-dependent behavior of anti-inflammatory activity and highest antibacterial activity against *E. coli* (zone diameter – 15 mm). However, none of the extracts exhibited antibacterial activity against *S. aureus*. Phytochemical investigations of extracts indicated the presence of alkaloids, flavonoids, phenols, tannins, saponins, terpenoids, glycosides and steroids. Significant results elicited by the combination of plant parts confirm that *M. figo* is a medicinal plant which can be used to develop novel anti-inflammatory agents.

Keywords: Antibacterial activity, Anti-inflammatory activity, *Magnolia figo,*

Introduction :

Use of traditional herbal and folk medicines is becoming more popular and globally accepted nowadays. Therefore, it is necessary to seek their medicinal properties and ascertain their therapeutic properties. 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 (Megha G. *et al*., 2013).

Inflammatory and infectious diseases are the most prevalent conditions leading to poor quality of life (Oz, 2017). The commonly used drugs for the management of inflammatory conditions are nonsteroidal anti-inflammatory drugs (NSAIDs) which have several side effects especially gastric irritation leading to the formation of gastric ulcers. Instead of side effects causing NSAIDs, the rich wealth of plant kingdom has been used to

represent a novel source of compounds with antiinflammatory activities (Chatterjee *et al*., 2012). Bacterial infections are the most common cause of inflammatory conditions and having a strong relationship that leads to find substituents that elicit both antibacterial and anti-inflammatory effects (Park *et al*., 2004). 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 (Valgas *et al*., 2007).

Magnolia figo is a plant belongs to Magnoliaceae family rich with secondary metabolites like alkaloids, polyphenols, tannins. Hence *M. figo* was selected and in *vitro* anti-inflammatory and antibacterial activity of methanolic extracts of leaves, roots and combination of both leaves and roots extracts of *M. figo* was evaluated in this study.

Methodology:

About 800 g of each matured, fully expanded leaves and roots of *M. figo* were collected in fresh condition at day time. Selected plant materials were thoroughly cleaned using running tap water and air-dried until a constant weight was obtained. The dried leaves were ground well to obtain fine powder form. Well dried and blended powder samples of each plant material were taken for the extraction procedure. The methanolic extract was obtained by cold maceration with 1000 mL of 80% methanol. Erlenmeyer flask was used to obtain a hydro alcoholic crude extract and it was stirred for 7 days at room temperature. The alcohol was distilled off and concentrated to a dry residue by evaporating the water from the filtrate using a rotary evaporator under reduced pressure.

For the anti-inflammatory study, plant samples were compared with diclofenac sodium under the same concentration. The dilution series (1000, 500, 250, 125, 62.5, 31.25, 15.625, 7.8125, 3.9, 1.95, 1, 0.5 µg/mL) of reference drug sample and the plant extracts were prepared. A similar volume of double distilled water was used as negative control. This process was carried out by using ELISA plate reader. A flat bottom ELISA plate which has 96 wells where each well consisted 300µl of reaction mixture was used for the evaluation. The mixtures were placed in an incubator at 37 °C (37±2°C) for 10 to 15 minutes. Denaturation process was induced by increasing the temperature gradually up to 57 °C in the laboratory oven. Samples were allowed to cool down to room temperature at 30 °C. After cooling down, the absorbance was measured at 660 nm using ELISA plate reader. The percentage inhibition of protein denaturation for each sample was calculated by using the absorption readings according to equation 100*[Vt/Vc-1] where Vt = absorbance of test sample and Vc = absorbance of control.

In the antibacterial studies, a serial dilution was prepared by re-dissolving crude extract in DMSO starting from 1500 µg/mL filtrate up to 250 µg/ml. Cylinder plate method was used to evaluate the antibacterial activity. DMSO was used as negative control while gentamicin was used as positive control. Mueller-Hinton agar was used as the culture media to determine antibacterial activity of *Staphylococcus aureus* (ATCC® 25923™) and *Escherichia coli* (ATCC® 25922™). The antibacterial activity was examined in triplicate for each sample and the diameter of the inhibition zone (in mm) for the extracts against the above-mentioned bacterial strains was measured and recorded.

The two extracts which were prepared by using only roots and combination of both leaves and roots were subjected to phytochemical analysis to detect the availability of the following secondary metabolites; flavonoids, carbohydrates, tannins, saponins, alkaloids, glycosides, phenols, terpenoids, amino acids and proteins and steroids.

Results and discussion Anti-inflammatory results:

Percentage inhibition of each plant extract and reference drug (diclofenac sodium) is summarized in Table 1. Data were calculated using the absorbance readings and represented as mean ± SEM. According to the results, combination extract showed the highest percentage inhibition compared to the other two extracts.

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
0.5	0.216 ± 0.23	10.891 ± 3.6	18.364 ± 18.6	11.614 ± 0.2

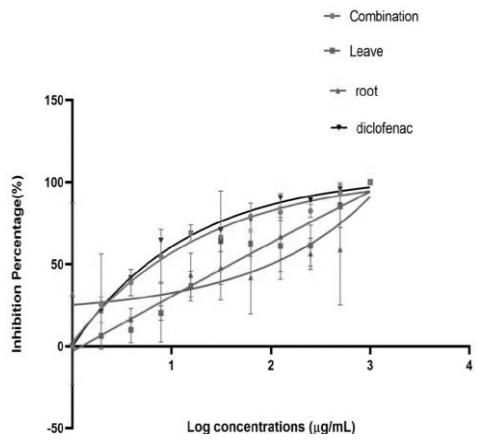

Figure 1 - Dose-response curves for anti-inflammatory properties of the M. figo plant leaves, roots and combination extracts and reference drug (diclofenac sodium) based on inhibition percentage.

Percentage inhibition data was used to calculate the dose response curve and the IC_{50} values for each extract. According to the dose-response curves, the combination extract and leaves extract showed highest potency compared to diclofenac sodium. The root extract showed lower potency compared to diclofenac sodium. Reference drug exhibited a higher IC_{50} value and a higher R^2 value than *M. figo* combination extract. The curve of the combination showed a similar pattern to that of the reference drug.

Figure 1 shows that, with the increasing log concentrations of *M. figo* leaves extract, percentage inhibitions were also increasing. A positive strong correlation (r^2=0.8307) between log concentrations and inhibition percentages of leaves extract was shown with an IC50 value of 4.132 µg/mL. Reference drug exhibited a higher IC50 value (4.337 µg/mL) and a higher R-square (r^2=0.9220) value compared to that of M. *figo* leaves extract.

With the increasing log concentrations of M. *figo* roots extract, percentage inhibitions were also increasing. A positive moderate correlation (r^2=0.4422) between log concentration and inhibition percentages were shown with 6.519 µg/mL IC50 value. Reference drug exhibited a lesser IC50 value compared to that of *M. figo* roots extract but higher R-square value than that of the roots extract.

It also showed that, with the increasing log concentrations of M. *figo* combination extract, percentage inhibitions were also increasing. A positive moderate correlation (r^2=0.5684) between log concentration and percentage inhibitions were shown with 1.819 µg/mL IC50 value. Reference drug exhibited a higher IC50 value and a higher R-square value than M. *figo* combination extract. The curve of the combination showed a similar pattern to that of the reference drug.

According to the Figure 1, combination extract and leaves extract showed higher potencies compared to diclofenac sodium. Roots extract showed lower potency compared to diclofenac sodium. (Details are given in the Table 2)

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-square	0.8307	0.4422	0.5684	0.9220
P value	<0.0001	<0.0001	<0.0001	<0.0001

Calculation of diclofenac sodium equivalents and milligrams of leaves, roots and combination extracts of *M. figo* reveals that anti-inflammatory activity of leaves of *M. figo* was found to be 1.0496 g diclofenac equivalents / gram (g) of the extract, anti-inflammatory activity of roots of *M. figo* was found to be 0.6653 g diclofenac equivalents / gram (g) of the extract and anti-inflammatory activity of roots of *M. figo* was found to be 0.6653 g diclofenac equivalents / gram (g) of the extract.

Antibacterial study results

The results of antibacterial activity screening are summarized in Table 3 and Table 4.

Table 3 - Antibacterial effect of methanolic leaves, roots and combination extracts of M. figo against E. Coli.

I → Concentrations Zone of inhibitions of *Magnolia figo* plant parts (µg/mL)

	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

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

II → Concentrations Zone of inhibitions of *Magnolia figo* plant parts (µg/mL)

	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)

Dose-response curves of methanolic leaves, roots and combination extracts of *M. Figo* against *E. Coli* is given in Figure 2.

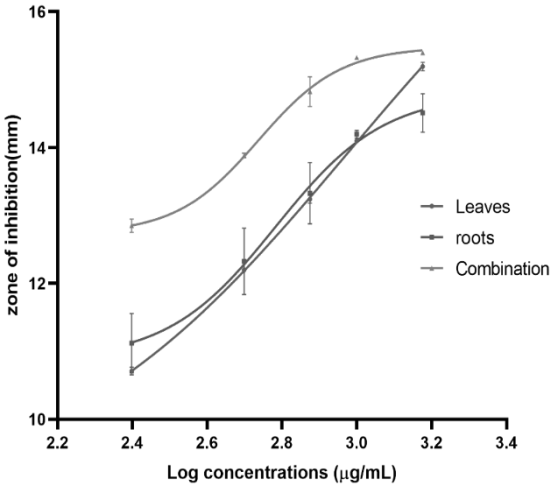

Figure 2 - Dose-response curves of methanolic leaves, roots and combination extracts of M. figo against E. coli

Results of our study show that the methanolic combination extract exhibited highest zone of inhibition (15.30 mm) against gram-negative

E. *coli.* Roots extract of *M. figo* exhibited lowest inhibition *(*14.45 mm*)* against gram negative *E. coli.* Accordingly, concentrations of the extracts have shown a positive correlation with zone of inhibition against E. *coli with* R^2 values equal to 0.99. Analysis of the data obtained from dose response study *(*figure 2*)* reveals that, the highest EC_{50} value *(*946.5 µg/mL*)* against E. *coli* is exhibited by the methanolic leaves extract whereas lowest EC_{50} value *(*536.2 µg/mL*)* against E. *coli* was obtained from methanolic combination extract. According to all these results, methanolic extracts of leaves, roots and combination of *M. figo* have shown positive antibacterial response against gram-negative bacteria *E. coli* and negative antibacterial response against gram-positive bacteria namely *S. aureus.* This indicates that leaves, roots and combination of *M. figo* extracts possess gram-negative antibacterial spectrum. It is advantageous to discover a novel antibacterial medicine to overcome the antibiotic drug resistance which is a problem at present.

The highest effect of anti-inflammatory activity was shown by the combination extract of *M. figo* followed by its leaves extract, 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. Variable presence of phytoconstituents in different parts of the plant such as roots and leaves might have been the reason for the above observations.

Results of phytochemical profile of methanolic extracts of roots and combination of leaves and roots of plant *M. figo* are expressed in table 5. The results are exhibited as the presence and the absence of bioactive compound (+) and (-) respectively and are given in Table 5.

Table 5- The results of the phytochemical analysis

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	+	+++
Amino acids and proteins	Ninhydrin test	+++	+++
Terpenoids	Salkowski test	-	+
Steroids	Liberman Burchard test	++	+

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

According to the findings of phytochemical studies, 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 have caused the synergistic effect in the combination extract.

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 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). Synergistic effect may be due to the secondary metabolites present in *M. figo* plant. Further studies are necessary to

ascertain the mechanism and the active constituents responsible for the anti-inflammatory and antibacterial activities of the methanolic extracts of plant parts of *M. figo*.

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