Research Article
CODEN (USA): IJPLCP
[Krithiga & Jayachitra, 3(9):Sep., 2012]
ISSN: 0976-7126

INTERNATIONAL JOURNAL OF PHARMACY & LIFE SCIENCES

Antioxidant and antibacterial study on *Coleus aromaticus* and *Lawsonia inermis*

N. Krithiga and A. Jayachitra*
Department of Plant Biotechnology, School of Biotechnology, Madurai Kamaraj University, Madurai, (TN) - India

Abstract

The Indian traditional medicine has a lot of potentiality to cure many human infections by using medicinal plants like *Coleus aromaticus* and *Lawsonia inermis*. For the present study these two plants were screened to detect the presence of the active metabolites like alkaloids, sugar, saponin, aminoacids, tannin and glycosides . The antimicrobial activity of *Coleus aromaticus* and *Lawsonia inermis* was analysed against different pathogens namely *Aeromonas hydrophilia, Eschericia coli, Edverchila tarda, Enterobacter aerogens, flavobacterium* by using alcoholic and water extracts. The results showed that the alcoholic extracts were taken in the concentrations 75 and 100 µg /ml of both the plants were effective in antimicrobial activity among these two plants *Lawsonia inermis* showed better activity.

Key-Words: Traditional medicinine, Anti-microbial activity, *Coleus aromaticus, Lawsonia inermis*

Introduction

Indian Material Medica includes about 2000 drugs of natural origin almost all of which are derived from different traditional system and folklore practices[1]. Antimicrobial activity of plants can be dedicated by observing the growth response of various micro organisms to those plant tissues or extracts, which are placed in contact with them. Many methods for detecting such activity are available, but since they are not equally sensitive or even based on the same principle, the results obtained will also be influenced by the method selected and the microorganisms used for the test. It is clear that biological evaluation in general can be carried out much more efficiently on water-soluble, nice crystalline screening of plant extracts have been surveyed by several authors [2,3].Henna (*Lawsonia inermis* L.) is a small shrub frequently cultivated in India, Persia, and along the African coast of the Mediterranean sea. powdered leaves of this plant, in the form of a paste, are used both as a cosmetic and as a cosmetic and as a remedy for boils, wounds, and some my cotic infections in certain countries of the Middle east.

*** Corresponding Author**
E.mail: jchitra21@gmail.com
Ph. No.: +0452-2458273

Material and Methods

Botanical description

Coleus aromaticus is a small plant with certain features that resemble a climber. Its stem is hairy, fleshy and attains a height of 1-3 feet. Leaves are 1-2 inch in length, roundish, dentate, hairy, thick and fleshy resembling heart. Flowers are small and blue or purplish in color with four petals, and it flowers in early summer.

Family**:** Lamiaceae
Genus: *Coleus*
Species: *aromaticus*
Botanical name**:** *Coleus aromaticus*

Lawsonia inmeris is much branched, deciduous, glabrous, sometime spinescent shrub or small tree with grayish brown bark, attaining a height of 2.4-5 m. It is cultivated as a hedge plant throughout India, and as a commercial crop in certain states of India for its dye.

Family**:** Lythracae
Genus: *Lawsonia*
Species: *inmeris*
Botanical name**:** *Lawsonia inmeris*

Collection of Plants samples

Locally available plants were selected for the study. The leaves of *Coleus aromaticus* and *Lawsonia inermis* were collected washed with water to remove soil and other adhering dirt particles. Then the leaves made into pieces with stainless steel knife and completely dried under shade (indoor). The air-dried leaves were

then coarsely powdered and used for extraction process.

Preparation of solvent extract

For preparation of extracts, successive solvent extraction method was followed using soxhlet apparatus. The dried and powered leaves of *Coleus aromaticus*, *Lawsonia inermis* (45 gm) were packed in the body of soxhlet extractor. The concentrated compounds after dryness were collected and used for various phytochemical analyses and for antibacterial studies.

Screening of Phytochemical Compounds

The various solvent extracts of the coarse powder of leaves of *Coleus aromaticus* and *Lawsonia inermis* were subjected to phytochemical tests for the identification of various active constituents, using the methodology followed[4]. The following major pharmaceutically valuable phytochemical compounds were analyzed.

Detection of alkaloids

A few drops of dilute hydrochloric acid were separately treated with 1 ml each of various extracts. Then it was filtered and the filtrates were treated with Mayer's reagent. The formation of cream precipitate confirmed the presence of alkaloids.

Detection of flavanoids

Take Five ml each of the various extracts was separately dissolved with 1 ml of alcohol was added with a few drops of neutral ferric chloride solution. Formation of blackish red colour indicated the presence of flavanoids.

Detection of phenols

Take One ml of the various extracts were dissolved in 5 ml of alcohol and treated separately with a few drops of neutral ferric chloride solution. The change in colour indicated the presence of phenols.

Detection of protein and free Amino Acids

Take 5 ml each of various extracts were dissolved in 5 ml of water separately and were subjected to Ninhydrin Test. Change in colour indicate the presence of protein.

Detection of saponins

To One ml each of the various extracts were separately mixed with 20 ml of distilled water and then agitated in a graduated cylinder for 15 min. The formation of foam indicated the presence of saponins.

Detection of phytosteroids and terpenoids

To Five ml each of various extracts were dissolved in 5 ml of Chloroform separately (stock solution). Then they were subjected to Liebermann – Burchard test. To one ml each of the stock solution, a few drops of acetic anhydride and 1 ml of concentrated sulphuric acid were added fro the sides of the test tubes and allowed to stand for 5 min. formation of brown ring at the junction of the two layers and the upper layer turned green indicated the presence of pgytostreoids and terpenoidal sapogenins.

Detection of tannins

To Five ml each of the various extracts were dissolved in minimum amount of water separately and filtered. The filtrates were taken separately and added a few drops of aqueous basic lead acetate solution. Formation of reddish brown precipitate indicated the presence of tannins.

Detection of sugars

To Five ml each of the various extracts were dissolved separately in distilled water, filtered and then subjected to Fehling's test. A small portion of the various filtrates was treated with 1 ml of Fehling's solution I and II and then heated gently. The formation of reddish brown color indicated the presence of sugars.

Detection of glycosides

Another portion of the extracts was hydrolysed with hydrochloric acid for few hours on the water bath and the hydrolysates were subjected to legal's test to detect the presence of different glycosides. To the hydrolysate 1 ml of pyridine and few drops of sodium nitro prusside solution were added and then it was made alkaline with sodium hydroxide solution. The appearance of pink to red color showed the presence of glycosides.

Antibacterial Study

The antimicrobial assay was done by the zone of inhibition at various concentrations (50, 75, 100µg/ml) of the various solvent extracts of and with the known concentration of commercial antibiotic compounds.

DPPH photometric assay

Principle

The ability of the leaf extracts to bleach DPPH can be quantified using a spectrophotometric assay, the extent of scavenging causing a proportionate change in the absorption at 518nm.

Reagents

DPPH (0.3 mM in methanol) , Methanol

Procedure

An exact amount (0.5ml) of the methanolic solution of DPPH was added with 20 µl of the *Lawsonia inmeris* and *Coleus aromaticus* leaves (corresponding to 4mg) and 0.48ml of methanol, and allowed to stand at room temperature for 30 minutes. Methanol served as the blank. After 30 minutes, the absorbance was measured at 518nm and converted into percentage radical scavenging activity as follows:

$$\text{Scavenging activity (\%)} = \frac{A_{518}\,[\text{sample}] - A_{518}\,[\text{blank}]}{A_{518}\,[\text{blank}]} \times 100$$

Methodology

The required amount of substances were weighted accurately and suspended in 100ml of distilled water and mixed thoroughly. The mixture was boiled and agitated to dissolve completely. This was then sterilized at 121^{O}C for 15 min. The pH of the medium was adjusted to 7.4 using 0.1 N NaOH. Anti bacterial property of solvent extracts of leaf was studied against the bacterial strains.

Screening of antibacterial activity

Disc Method

The molten bacterial media was poured into sterilize Petri dishes separately which were labeled appropriately using aseptic technique. A moisten swab with the broth culture of microorganisms was spread over the surface of agar plate by mounting ball and froth in all directions for antibacterial activity commercial antibiotic ciproflaxacin was used. The extracts of various solvents were taken in separate containers. Sterile disc that were soaked in various solvent extracts were then placed in the inoculated Petri plates. The Petri dishes were then incubated at 37^{O}C for 24 hrs in an inverted position. The inhibition zone of microbes was observed and were measured. Replicated study of each sample was conducted. Simultaneously control was also performed

Diffusion method

The molten bacterial media was poured into sterile Petri dishes separately which were labeled appropriately. The culture were spread on the plate, using swab. Wells were cutting the cork borer. 60 µl of sample aliquots were pipetted into the well. The plates were incubated at 37^{O}C for 24 hours. Zone of inhibition in diameter was measured by a scale. Replicated study of each sample was conducted. Simultaneously control was also performed.

Results and Discussion

The alcohol and water extract of C*oleus aromaticus* and *Lawsonia inermis* showed the presence of alkaloid, sugar, aminoacids, tannin, and glycosides (Table I).The antioxidant activity was analysed using DPPH method (Figure 1). To study the antibacterial activity the experiments were done using the alcohol and water extracts of the leaves of the plants *Coleus aromaticus* and *Lawsonia inermis*. The concentration chosen were 50µg ,75 µg and 100 µg/ml.The antibacterial activity was tested against the pathogenic organism *Aeromonas hydrophilia, Escherichia Coli, Enterobacter aerogens, Flavobactrium* and *Edverchila tarda.* The experiment was performed using two methods such as Disc method and Diffusion method. The results for antibacterial activity of the alcohol and water extracts of *Coleus aromaticus* using Disc Method showed that the zone of inhibition was higher concentrations of 100 mg/ml and 50 mg/ml than the control both in alcohol extract and water extract (Figure 2a). It was observed that zone of inhibition was seen for all the organisms of both extracts. A higher zone of inhibition was seen for the organism *Enterobacter Aerogens, Aeromonas hydrophilia and Edverchila tarda*. In the well diffusion method a higher zone of inhibition was observed in 75 mg/ml and 100 mg/ml of leaf extracts of alcohol and water (Figure 2b). The inhibition zone was higher for all bacterial cultures in alcohol and water extracts. A higher activity was seen for *Aeromonas hydrophilia, Enterobacter aerogens, Escherilia coli,* and *Edverchila tarda*. Figure 3 a and b shows the overall percentage of activity 0.25% in 75 mg alcohol extract and 0.34% in 100 mg alcohol extract.The overall activity was slightly lower in the water extracts. It was 0.15% in 75 mg and 0.19% in 100 µg. The overall percentage activity was 0.23% for 75 mg alcohol extract, 0.26% for 100 mg alcohol extracts. For the water extracts it was 0.17% in 75 mg extracts and 0.19 in 100mg extract.

Figure 2c and 2 d shows the result for antibacterial activity of the water and alcohol extracts of *Lawsonia inermis* using Disc Method also showed that the zone of inhibition was higher in 75 mg/ml and 100 mg/ml extracts.A higher zone of inhibition was observed in all bacterial cultures in *Aeromonas Hydrophilia, Eschericia coli*, *Edverchila tarda*, *Enterobacter aerogens, Flavobacterium*. The Diffusion method experiment for *Lawsonia inermis* showed a higher activity in 75 mg/ml and 100 mg/ml extracts.A higher zone of inhibition was observed in the alcohol extracts for the pure culture of all the experimental micro organisms. In the water extracts also a higher activity was observed for 75 mg/ml and 100 mg/ml water extracts. The zone of inhibition was also higher for all the bacterial cultures. The activity observed was higher for all organisms. It was very high for *Eschericia coli, Aeromonas Hydrophilia, and Edverchila tarda*. Figure 3 c and d shows the overall percentage of activity was 0.45% in 75 mg extracts and 0.52% in 100 mg extracts for alcohol extracts. However the zone of inhibition was lower in water extracts. It was 0.17% in 75 mg and 0.19% in 100 mg of water extracts. The overall percentage of activity was 0.19% in 75 mg extract and 0.41% in 100 mg extract.

Discussion

In our study for antibacterial study the aqueous and organic extracts showed different results. The ethanolic leaf extract showed greater activity. Similar findings and conclusion were drawn[5].The antioxidant

property of the two plants were effective. The experimental plants *Coleus aromaticus* and *Lawsonia inermis* exhibited effective antibacterial activity.The effectiveness of these plants in net due to one main active constituent but due to a combined action of other secondary metabolites .The Secondary metabolites which have been observed in our experimental plants include alkaloids, Tannin and glycosides, which are classified or antimicrobial compounds[6].It could be also observed that the performance of the plants were almost same in both methods – Disc and well diffusion method. Among the two experimental plants the alcoholic extracts *Lawsonia inermis* was observed to b exhibiting better antimicrobial activity. This probably explain the reason why this plant in too often used in colouring the fingers and hairs of Indians, in the form of henna and has also become a traditional culture among women in Tamilnadu. *Coleus aromaticus* and *Lawsonia inermis* due to its antibacterial activity can be a cure for common diseases like cold, asthma, Cough, Severe throat and nasal congestion. *Lawsonia inermis* can be a cure for eczema itching, skin infections and ringworm. Wounds and burn wound infection [7]. *Lawsonia inermis* has naphthoquinone or hennotannic acid which is considered to be playing a role in antibacterial activity. But the Isolation, and characterization of the active principles in these plants needs be done further. However till now all antimicrobial substances from higher plants have been found either to be toxic to animals or not competitive therapeutically, with the products of microbial origin due to their low potency and narrow spectrum. Quality control of herbal drugs[8].Therefore no antimicrobial compound from a higher plant has yet come into significant clinical use. However rsearch in taking place in the hope of finding out effective antimicrobials, to treat human infections. Since the screening methodology for detection of such agents, their isolation from the plants and successive structure activity study are easy to perform there are grounds for optimism in the field of herbal medicine. This optimism comes true the current search for substitutes to antibiotics may well come true one day.

References

1. Narayana D.B.A., JKatayar C.K. and Brindavanam N.B. (1998). Original system ; search, re-search. IDMA Bulletin 29(17): 413-416.
2. Janssen A.M., Scheffer J.J.C. and Baerheim S.A. (1987). Planta medica 53: 395-398.
3. Rios J.J., Rcio Mc. And Villard A. (1988). Jr. of Ethnopharmacology 23: 127-149.
4. Malcom and sofowora (1969). Antimicrobial screening of extracts of medicinal plant , Jr. of Micro biotech 7: 556-557.
5. Krishna K.T., Ranjini C.E. and Sasidharan V.K. (1997). Antibacterial and antifungal activity of secondary metabolites from some medicinal and other common plant species journal of life science 2; 14-19.
6. Rojas R., Bauer J., Fernanddez I., Albang and Locko, (2003). Antimicrobial activity of selected perurian medicinal plants. J. ethnopharmacol 88(23) :199-204.
7. Muhammed H.S. and Muhammad S. (2005). The use of *lawsonia inermis* Linn. (Henna) in th management of burn wound infection. Jr. for microbial biotech 15-17.
8. Mukherjee pulok K. (2001). Evaluation of Indian traditional. J. Drug information, USA 35,:631-640.

Table I: Phytochemical screening of extracts

Extracts	alkaloid	sugar	saponins	amino acids	flavanoids	steroids	phenols	tannin	glycosides
Ethanol *(C.a)*	+	++	++	+	-	-	-	+	+
Water *(C.a)*	++	+++	++	++	-	-	-	+	++
Ethanol *(L.i)*	++	++	++	+	-	-	-	+	+
Water *(L.i)*	++	+++	++	++	-	-	-	+	++

(+): Presence of the Compound, (+++) (++): Increased Concentration of Compounds, (-): Absence of Compound, C.a *Coleus aromaticus,* L.i *Lawsonia inermis*

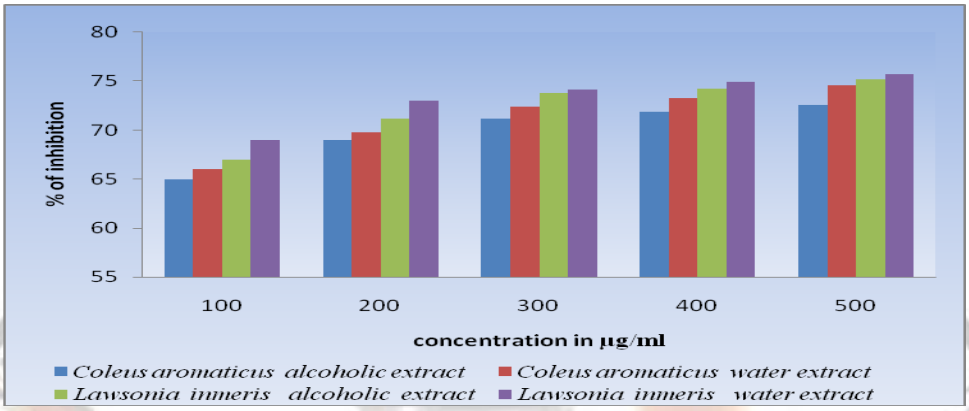
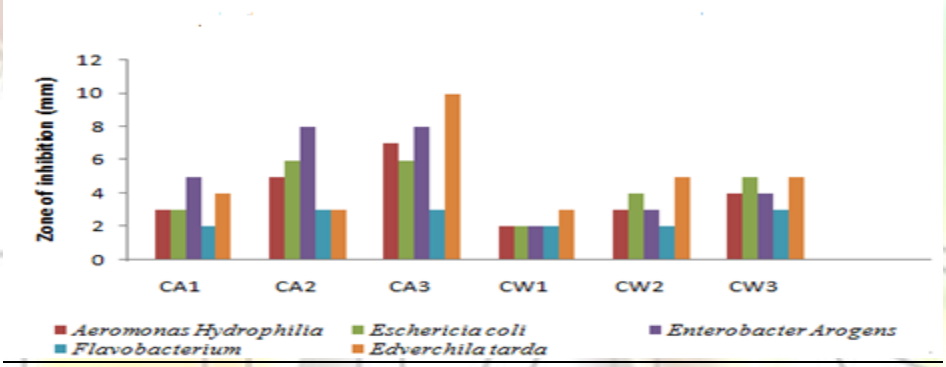
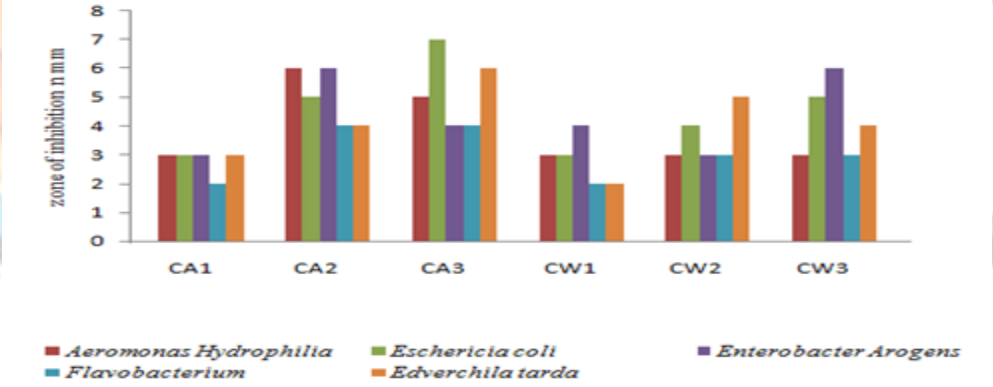


Fig. 1: Total antioxidant activity by DPPH method



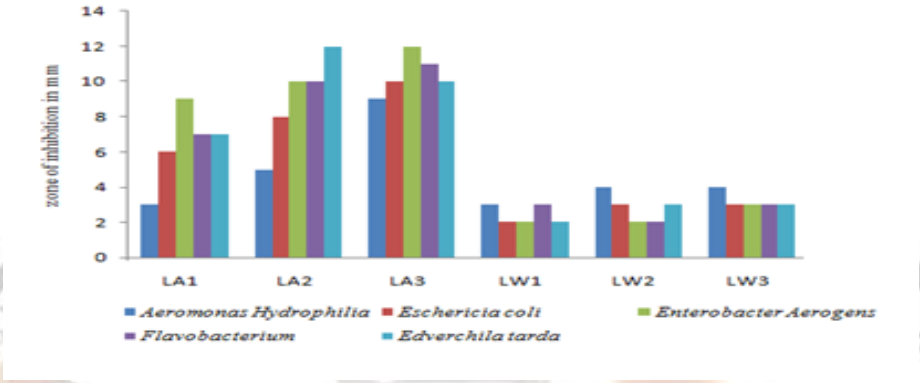
CA 1 - *Coleus aromaticus* in Alcohol (50 µg) CW 1 - *Coleus aromaticus* in Water (50 µg)
CA2 - *Coleus aromaticus* in Alcohol (75 µg) CW 2 - *Coleus aromaticus* in Water (75 µg)
CA3 - *Coleus aromaticus* in Alcohol (100 µg) CW 3 - *Coleus aromaticus* in Water (100 µg)

Fig. 2a: Antibacterial activity of *Coleus aromaticus*(alcoholic and water extract) disc diffusion method



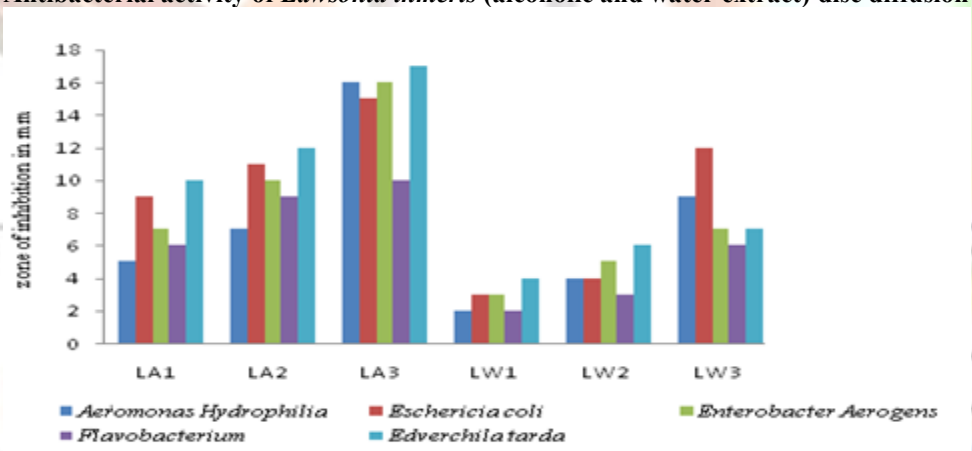
CA 1 - *Coleus aromaticus* in Alcohol (50 µg) CW 1 - *Coleus aromaticus* in Water (50 µg)
CA2 - *Coleus aromaticus* in Alcohol (75 µg) CW 2 - *Coleus aromaticus* in Water (75 µg)
CA3 - *Coleus aromaticus* in Alcohol (100 µg) CW 3 - *Coleus aromaticus* in Water (100 µg)

Fig. 2b: Antibacterial activity of (alcoholic and water extract) well diffusion method



LA1 - *Lawsonia inermis in Alcohol (50 mg)* LW1 -*Lawsonia inermis in Water (50 mg)*
LA2 - *Lawsonia inermis in Alcohol (75 mg)* LW2 -*Lawsonia inermis in Water (75 mg)*
LA3 - *Lawsonia inermis in Alcohol (100 mg)* LW3 -*Lawsonia inermis in Water (100 mg)*

Fig. 2c: Antibacterial activity of *Lawsonia inmeris* (alcoholic and water extract) disc diffusion method



LA1 - *Lawsonia inermis in Alcohol (50 mg)* LW1 -*Lawsonia inermis in Water (50 mg)*
LA2 - *Lawsonia inermis in Alcohol (75 mg)* LW2 -*Lawsonia inermis in Water (75 mg)*
LA3 - *Lawsonia inermis in Alcohol (100 mg)* LW3 -*Lawsonia inermis in Water (100 mg)*

Fig. 2d: Antibacterial activity of *Lawsonia inmeris* (alcoholic and water extract) well diffusion method

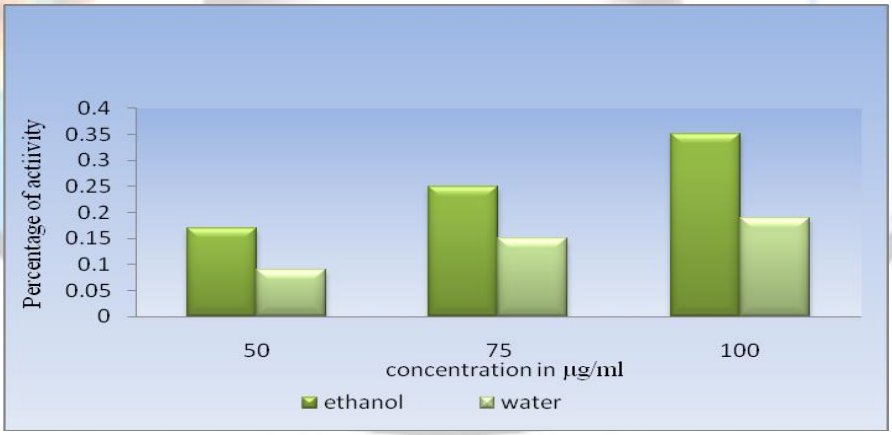


Fig. 3a: Percentage of antibacterial activity (disc) by *Coleus aromatius*

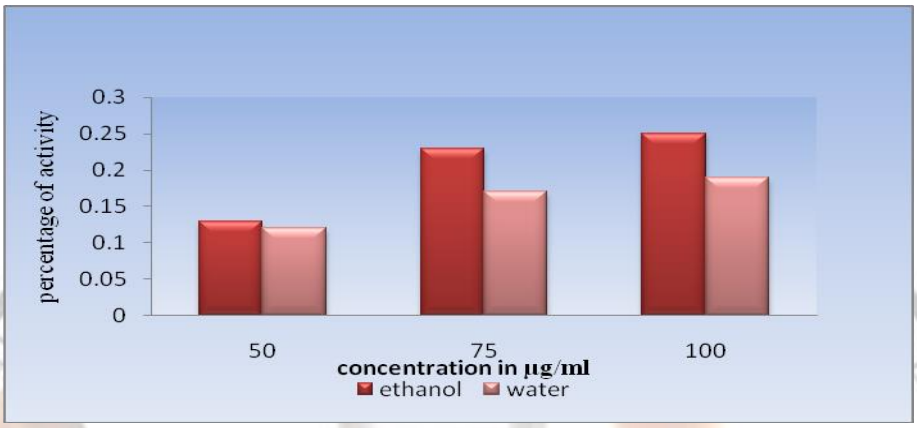


Fig. 3b: Percentage of antibacterial activity (well) by *Coleus aromatius*

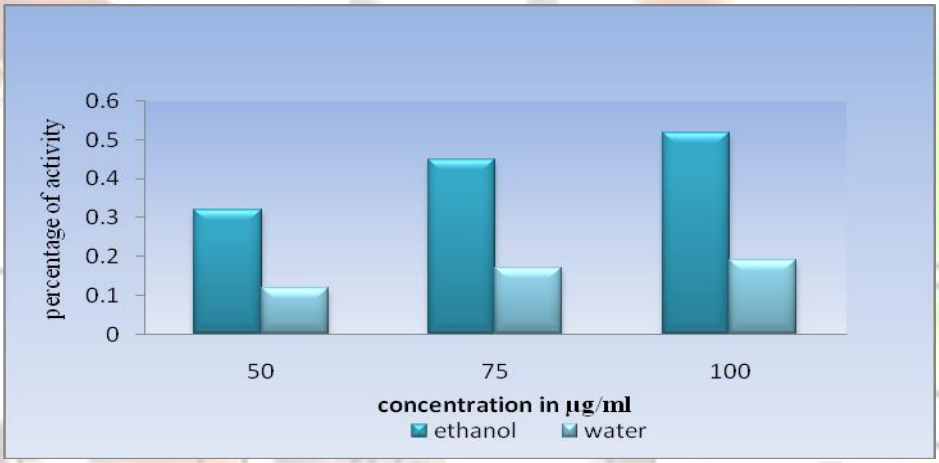


Fig. 3c: Percentage of antibacterial activity of *Lawsonia inmeris*