COMPARATIVE ANTIMICROBIAL AND HEMATOLOGICAL ACTIVITY OF DIFFERENT LEAVES AND RHIZOME EXTRACT OF *CURCUMA LONGA*

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ABSTRACT

In present study anti bacterial activity of three extract of *Curcuma Longa* i.e. 95% ethanolic leaf extract, Aqueous Leaf Extract, 95% ethanolic rhizome extract was monitored using disc diffusion method. Activity was determined by noting the zone of inhibition around the disc. A Gram negative & Gram positive bacterial strains (5 strains of each) used. Antibacterial activity of three extract of *Curcuma Longa* was checked against these bacterial strains. The study reveals that the 95% ethanolic leaf and rhizome extract at a concentration 12.5mg/ml were effective against Gram negative bacterial strain. 95% ethanolic leaf extract show better antibacterial activity then the rhizome extract against the *Enterobacter aerogens* and *Streptobacillus monaliformus* (Gram negative) and *streptococcus epidermis* (Gram positive) bacterial strains and show no activity against three Gram positive bacteria (*Streptococcus pyrogens, Streptococcus aureus*, and *Streptococcus pneunoniae*) and Gram negative (*Escherichia coli*) .Aqueous leaf extract exhibited no significant activity against the Gram negative and Gram positive bacterial strains. To drugs Antibiotic Cefotaxime and Amikacin used as a control for anti bacterial activity against Gram negative and Gram positive bacteria in the present set of studies.

KEYWORDS: *Curcuma Longa,* Hematological, Anti bacterial activity, Gram Negative and Gram Positive Bacteria. Disc Diffusion.

INTRODUCTION

Medicinal plants are important source for the verification of pharmacological effects and can be natural composite sources that act as new anti-infectious agents (Ushimaru *etal*., 2007). Different plant parts are used for medicinal purposes i.e., bulb, gel, leaves, roots, barks, peels

etc. The use of plants to treat illness is found throughout human culture (Anne-Catherine, 2007).

Curcuma longa (C. longa), a perennial herb, is a member of the Zingiberaceae family and has a Long tradition and Ayurvedic systems of medicine. Curcuminoids, a group of phenolic compounds isolated from the roots of C. longa, exhibited a variety of beneficial effects on health and has the ability to prevent certain diseases (Joe, B., M. 2004].*C. longa*, commonly known as 'turmeric', is widely used as a spice and colouring agent, and is well known for its medicinal properties It is cultivated primarily in Bengal, China, Taiwan, Sri Lanka, Java, peru, Australia & West Indies, Andhra Pradesh, Tamil Nadu, Karnataka, Kerla, Bihar & Assam. Turmeric held a place of honor in India's traditional Ayurvedic Medicines. . It contains a mixture of powerful antioxidant phytonutrients known as curcuminoids and inhibits cancer at initiation, promotion and progression stages of tumor development. It is a strong anti-oxidant, which supports colon health, exerts neuroprotective activity and helps to maintain a healthy cardiovascular system (Luthra *et al*., 2001).

The most resistant strains have typically been found in hospitals, particularly in intensive care units, where antibiotics are extensively used (Guilfoile, 2007). With the continuous use of antibiotics, there is an increasing resistance of microorganisms towards them. In addition to this problem, antibiotics are sometimes associated with adverse effects on host which include: hypersensitivity, depletion of beneficial gut and mucosal microorganisms, gastric disturbances, ototoxicity, nephrotoxicity, immunosuppression and allergic reaction (Lopez et al., 2001; Cosgrove et al., 2009). Due to the above reasons of using synthetic antibiotics and because there is a constant need for new and effective therapeutic agents (Bhavnani and Ballow, 2000; Singh and Jain, 2011), many researchers have focused on the investigation of an alternative antimicrobial drugs from natural products as a source of new bioactive molecules for treatment of infections (Cordell, 2000; Singh and Jain, 2011).

The continuous evolution of bacterial resistance to currently available antibiotics has necessitated the search for novel and effective antimicrobial compounds. Globally, plant extracts are employed for their antibacterial, antifungal and antiviral activities. (Odugbemi, 2006). In the indigenous system of medicine, turmeric, has the top most priority & enjoys the reputation as a remedy for a number of ailment including stoma chic, blood purifier, leprosy dropsy, purulant, opthalmia, pyrogenic. Infections, would healing & inflammations.

But the leaf of Turmeric plant is a waste product generated during post harvesting operations of turmeric crop. It is usually used as fuel in rural India to a small extent or as green manure the leaf is aromatic & contains essential oil.

In comparison to rhizome, turmeric leaf is hardly investigated for its bio medical activities. This prompted is to undertaken this study to observe the hematological and antibacterial activity of turmeric leaf against gram negative & gram positive microorganisms & to compare the activities with rhizome & their comparative study with other standard antibiotics.

MATERIAL AND METHOD

1. Extraction

1.1 Selection of the plant material

Based on the Antioxidant, Chemomodulatory, Antitumor, Antibacterial and Cytotoxic activity the plant has been selected for Immunomodulatory, Antioxidant and Antibacterial activity by using different part of the plant. Various active chemical constituent have been reported in *Curcuma Longa* which have Antioxidant, Immunomodulatory, Anticancer, Anti Inflammatory, Antiheptotoxic properties. But none of the I*n vivo and In vitro* study on *Curcuma Longa* leaves has been reported.

1.2 Identification collection and authentication of plant.

The leaves of *Curcuma Longa* were identified and authenticated from department of pharmacy BUB and collected locally from Jawaharlal Nehru cancer hospital Bhopal, sanjivani ayurveda T.T. nagar Bhopal.

1.3 Processing

1.3.1 Washing and cutting: the leaves were subjected to washing to remove all dust particles

1.3.2 Shade drying: The parts were further subjected to drying separately.

1.3.3 Comminution: The dry leaves were further subjected to size reduction by comminution for efficient extraction. The initial quantity of powder were taken and the powder were stored in dry and air tight container

1.4 Extraction protocol

1.4.1 Part used -   leaves

1.4.2 Solvent -95% ethanol, distil water

1.4.3 Method of extraction -cold maceration method

1.4.4 Extracts-95% ethanolic extract, aqueous extract

1.5 Preparation of extract

1.5.1 cold maceration method: about 100 gm of coarsely powdered, air dry leaf powder was macerated in a dry glass stoppered glass container the powered were soaked in the sufficient quantity of solvent (95% ethanol) in the ratio of (1:10) drug : solvent ratio for seven days in cool and dark place with occasional stirring after 7 days mixtures was strained. The strained liquid was filtered separately. The liquid were mixed and concentrated liquids were transferred to a tarred flat bottom dish and evaporated to dryness in hot air oven at 25 to 30 degree centigrade. The dried extract was cooled in desiccators and weighed. Same process was used with chloroform water as solvent to obtain aqueous extract. The percentage yield calculated for each extracts on the basis of fresh weight and dry weight.

2.1 Pharmacological studies

2.1.1Methodology

Assay for antibacterial activity 43

A. Micro organisms: microbial strains used for the test were *Klebsiella pneumoniae, Enterobacter aerogenes, Pseudomonas aeruginosa, Escherichia coli, Sreptobaccilus monaliformus* **(Gram (-) bacteria)**

Streptococcus pyogenes, Staphylococcus aureus, Staphylococcus epidermidis, Colestridium tetnii, Streptococcus pneumoniae **(Gram (+) bacteria)**

The bacterial strain obtained from the shivam pathology, Rewa medical collage and activity are always carried out under the observation of Sanjay Gandhi medical collage Rewa.

B. Medium: the media used the muleller hinton agar for bacteria testing.

B. Determination of antimicrobial activity Rhizome Extract was tested for antimicrobial activity by disc diffusion method. Sterile paper discs (6mm) were impregnated with reconstituted crude extract in different concentration (6mg, 12.5 mg and 25 mg / ml in 20% DMSO) and placed on the surface of media for bacteria and inoculated with the microbes. The sample was tested in triplicate. Disc containing same concentration of DMSO, 25 μg/disc of Cefotaxime and Amikacin were used as a solvent control and a positive control for bacteria. Agar plates containing bacteria were incubated at 370C for 24hrs. Inhibition zones

were recorded as the diameter of growth free zone, including the diameter of the disc, in mm at the end of the incubation period.

Immunization: after drug treatment, on 8th day all the rat were immunized with sheep RBCS (2 X 107 cells/rat) intraperitoneally and were later studies for the parameters given below to evaluate and compare the immunostimulant activity and effect on hematological parameter of *Curcuma Longa* with the market sample.

Haemotological profile : after 7th days of the administration of the extracts and after immunization on 7th and 15th days, blood samples were collected from individual animals of all the groups by heart puncture for haemotological parameter such as haemoglobin content, Leukocytes, Erythrocytes, Packed cell volume were determined by using cell counter.

Immune response against sheep RBC of different extracts/drug: after immunization on 7th and 15th days, blood samples were collected from individual animals of all the groups by heart puncture for haemglutination antibody (H.A) titre. In the other group the delayed type hypersensitivity (DTH) response to SRBC was determined.
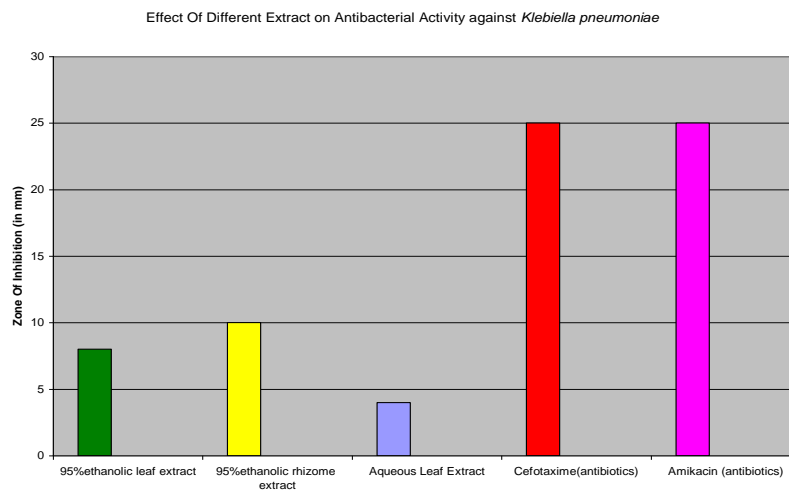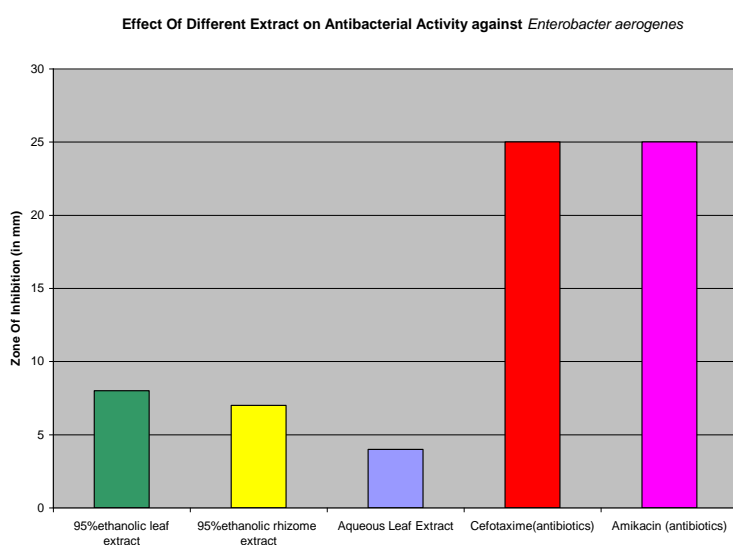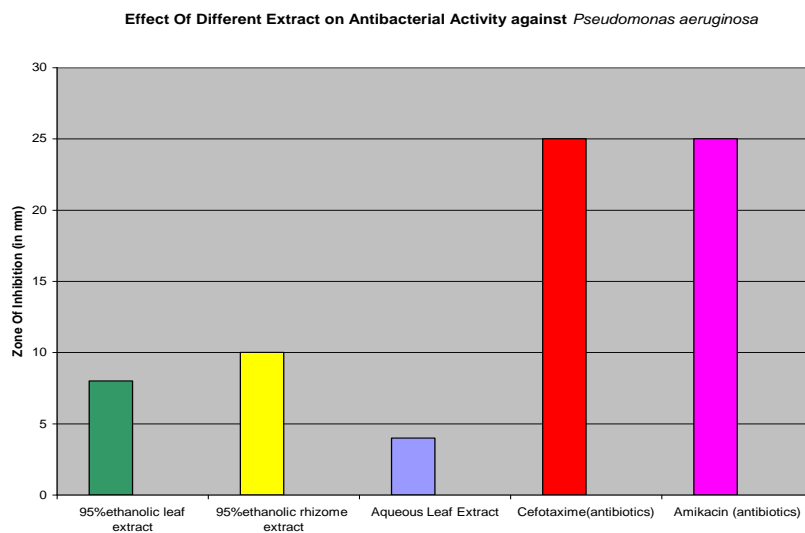
RESULT

Table1: Extraction of crude drug and respective yields on dry weight basis.

Plant part	Solvent	Method	1. %Yield
Leaf	Chloroform water	maceration	2. 13.6
Leaf	Alcohol	maceration	3. 14.76
Rhizome	Alcohol	maceration	4. 41.92

Antibacterial Activity

Table 2: Effect of different extract on Antibacterial Activity against Gram (-) Bacteria

Microorganisms	*Klebsiella pneumonia*	*Enterobacter aerogenes*	*Pseudomonas aeruginosa*	*E.coli*	*Sreptobaccilus monaliformus*
	Zone of inhibition(mm in diameter)				
Concentration mg/disc	12.5	12.5	12.5	12.5	12.5
95% ethanolic leaf extract	8	8	8	4	8
95% ethanolic rhizome extract	10	7	10	8	7
Aqueous leaf extract	4	4	4	4	4
Cefotaxime (antibiotics)	25	25	25	25	25
Amikacin (antibiotics)	25	25	25	25	25

Effect Of Different Extract on Antibacterial Activity against *Klebiella pneumoniae*

Figure1

Effect Of Different Extract on Antibacterial Activity against *Enterobacter aerogenes*

Figure2

Effect Of Different Extract on Antibacterial Activity against *Pseudomonas aeruginosa*

Figure3

Effect Of Different Extract on Antibacterial Activity against *Escherichia coli*

Figure4

Effect Of Different Extract on Antibacterial Activity against *Sreptobaccilus monaliformus*

Figure5

Table 3: Effect of different extract on Antibacterial Activity against Gram (+) bacteria

Microorganisms	*Streptococcus pyogenes*	*Staphylococcus aureus*	*Staphylococcus epidermidis*	*Colestridium tetnii*	*Streptococcus pneumoniae*
	Zone of inhibition(mm in diameter)				
Concentration mg/disc	12.5	12.5	12.5	12.5	12.5
95% ethanolic leaf extract	0	0	11	7	0
95% ethanolic rhizome extract	9	9	9	11	9
Aqueous Leaf extract	4	4	4	4	4

Cefotaxime (antibiotics)	25	25	25	25	25
Amikacin (antibiotics)	25	25	25	25	25

Effect Of Different Extract on Antibacterial Activity against *Streptococcus pyogenes*

Figure 6

Effect Of Different Extract on Antibacterial Activity against *Staphylococcus aureus*

Figure7

Effect Of Different Extract on Antibacterial Activity against *Staphylococcus epidermidis*

Figure: 8

Effect Of Different Extract on Antibacterial Activity against *Colestridium tetnii*

Figure: 9

Effect Of Different Extract on Antibacterial Activity against *Streptococcus pneumoniae*
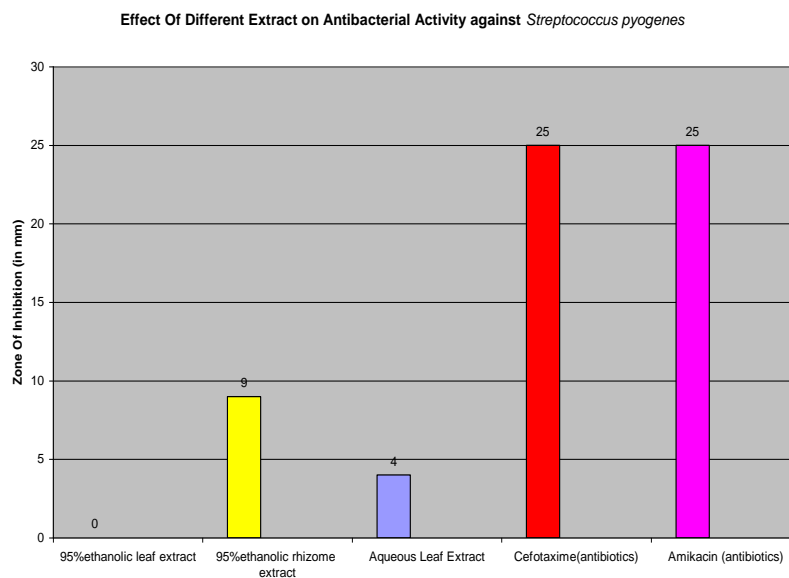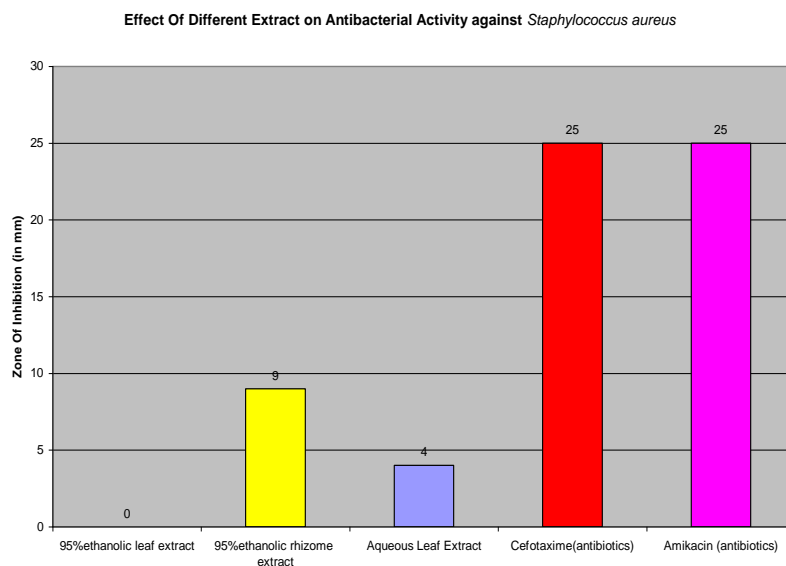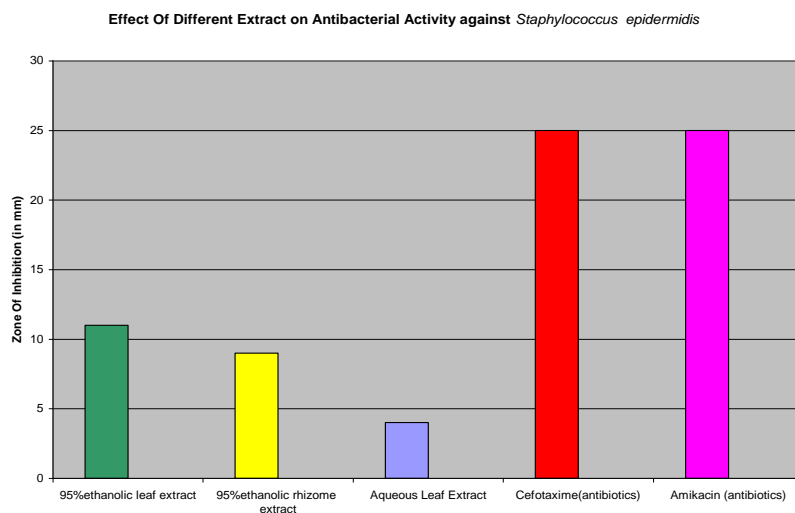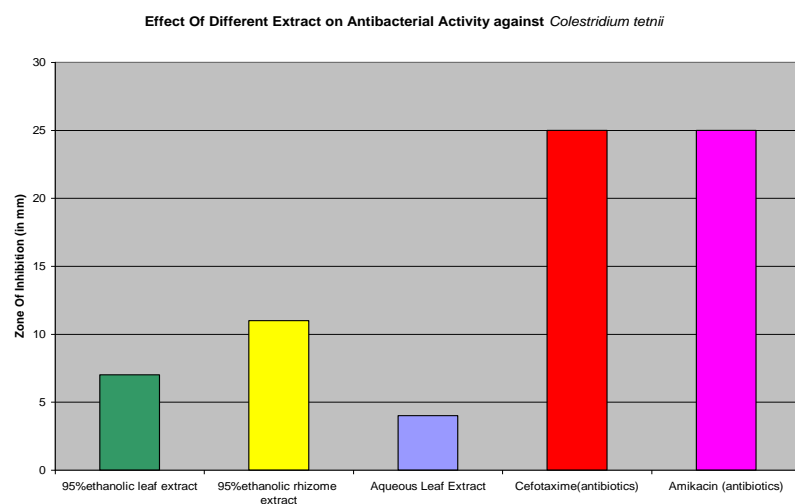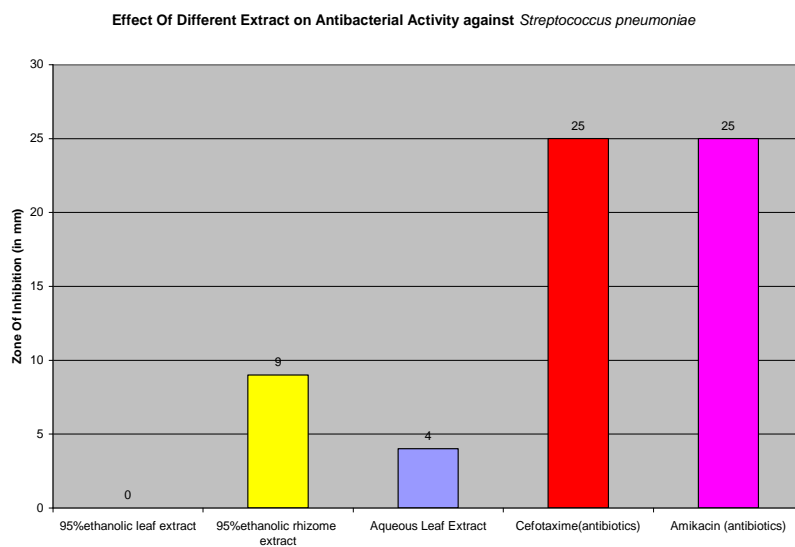
Figure: 10

Table 4: Effect of different extract on Haematological parameters Before Immunization and after 7 days of Extract treatment of rats

substance/exract	Dose (mg/kg)	Effect on Haemtological parameter (mean ±SD)						
	Oral	WBC 10^3/cu mn	RBC 10^6/cu mn	HGB %	N /100cells	L /100cells	M /100cells	E /100cells
Control	0.5%CMC	11.45± 70.71	3.97±0. 00	10.85 ±0.07	42.50±0 .70	53.50± 0.70	4.00±0. 00	1.00±0. 00
Standard (Reconia G)	120	10.65± 70.71	3.94±0. 03	10.94 ±0.03	52.00±1 .41	44.00± 1.41	3.50±0. 70	2.00±0. 00
95% ethanolic leaf Extract	500	12.87± 35.35	3.70±0. 071	9.35± 0.21	37.50±2 .12	58.50± 0.70	4.00±0. 00	1.00±0. 00
Aqueous Leaf Extract	500	10.83± 49.49	4.66±0. 04	13.35 ±0.21	72.00±0 .00	24.00± 1.41	3.50±0. 70	4.00±0. 00
95% ethanolic Rhizome extract	500	10.47± 35.35	4.82±0. 04	13.60 ±0.14	72.00±1 .41	22.50± 2.12	2.50±0. 70	4.00±0. 01

Table 5: Effect of different extract on Haematological parameters after 7 days of Immunization of rats

substance/exract	Dose (mg/kg)	Effect on heamtological parameter (mean ±SD)						
	Oral	WBC 10^3/cu mn	RBC 10^6/cu mn	HGB %	N /100cells	L /100cells	M /100cells	E /100cells
Control	0.5%CMC	11.65± 60.71	4.17±0.1 0	11.30 ±0.14	42.00± 1.41	53.50± 2.12	4.50±0. 70	2.50±0 .70
Standard (Reconia G)	120	10.82± 35.35	4.61±0.0 5	13.05 ±0.21	36.50± 2.12	60.00± 2.82	5.00±0. 70	2.50±0 .00
95% ethanolic leaf Extract	500	11.37± 106.06	3.97±0.0 3	10.65 ±0.28	31.50± 3.53	68.00± 1.42	3.50±0. 70	1.00±0 .00
Aqueous Leaf Extract	500	11.62± 35.35	4.30±0.0 2	10.65 ±0.07	72±1.4 1	49.00± 0.00	2.00±0. 00	5.00±1 .41
95% ethanolic Rhizome extract	500	11.26± 650.53	4.49±0.0 3	12.75 ±0.21	71.50± 0.70	23.50± 3.53	3.50±0. 00	7.00±1 .41

Table 6: Effect of different extract on Haematological parameters after 15 days of Immunization of rats

substance/exract	Dose (mg/kg)	Effect on heamtological parameter (mean ±SD)						
	Oral	WBC 10^3/cu mn	RBC 10^6/cu mn	HGB %	N /100cells	L /100cells	M /100cells	E /100cells
Control	0.5% CMC	10.63± 49.49	4.19±0. 28	11.50 ±0.15	42.00± 1.41	52.00±1. 41	4.50±0. 70	2.00±0 .00
Standard (Reconia G)	120	10.48± 162.63	4.28±0. 04	11.95 ±0.07	38.50± 0.70	57.50±2. 12	4.50±2. 14	1.00±0 .01

95% ethanolic leaf Extract	500	10.68± 120.20	3.63±0. 02	9.60± 0.42	36.50± 2.12	59.00±0. 01	3.50±0. 01	4.00±0 .00
Aqueous Leaf Extract	500	13.52± 35.35	4.34±0. 07	11.80 ±0.14	36±2.1 3	58.5±0.7 1	4.25±0. 21	3.50±0 .21
95% ethanolic Rhizome extract	500	96.75± 106.06	4.18±0. 01	11.50 ±0.00	52.00± 1.41	45.00±4. 24	4.50±0. 70	4.00±0 .01

Values are represented as mean ± SD (N=4)

WBC-White blood cell, RBC-Red blood cell, HGB-Hemoglobin,

N-Neutrophil, L-Lyphocyte, M-Monocyte, E-Eosinophill
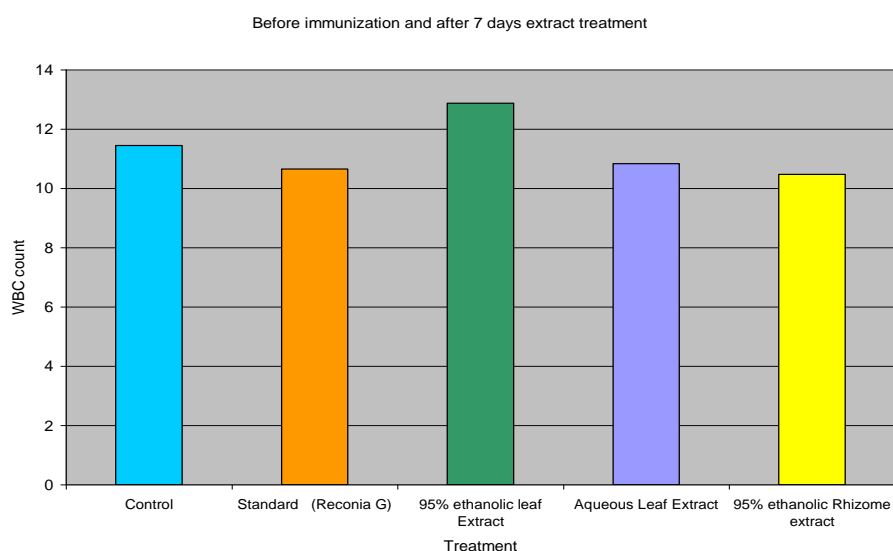
Figure: 11 effect of different extract of *Curcuma Longa* on WBC count (10^3/cu mm) after 7days of extract treatment and before immunization
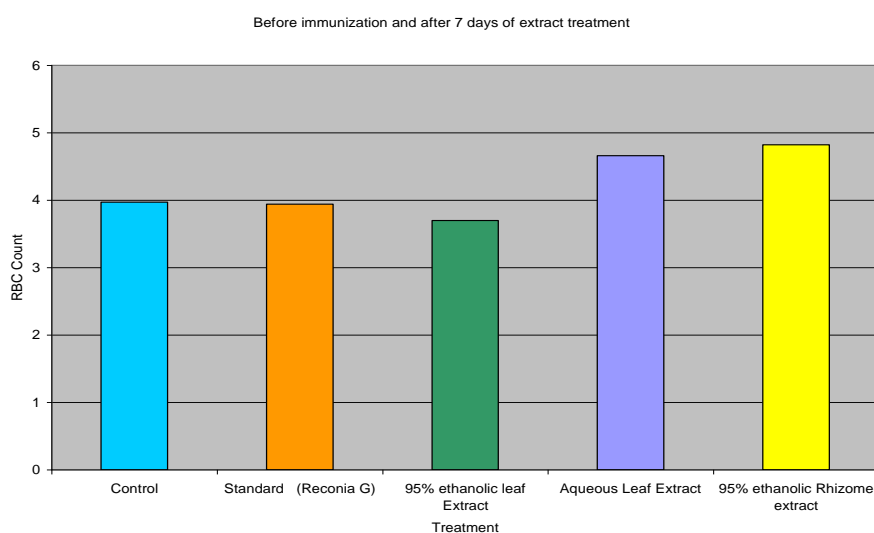
Figure: 12 effect of different extract of *Curcuma Longa* on RBC count (10^6/cu mm) after 7days of extract treatment and before immunization
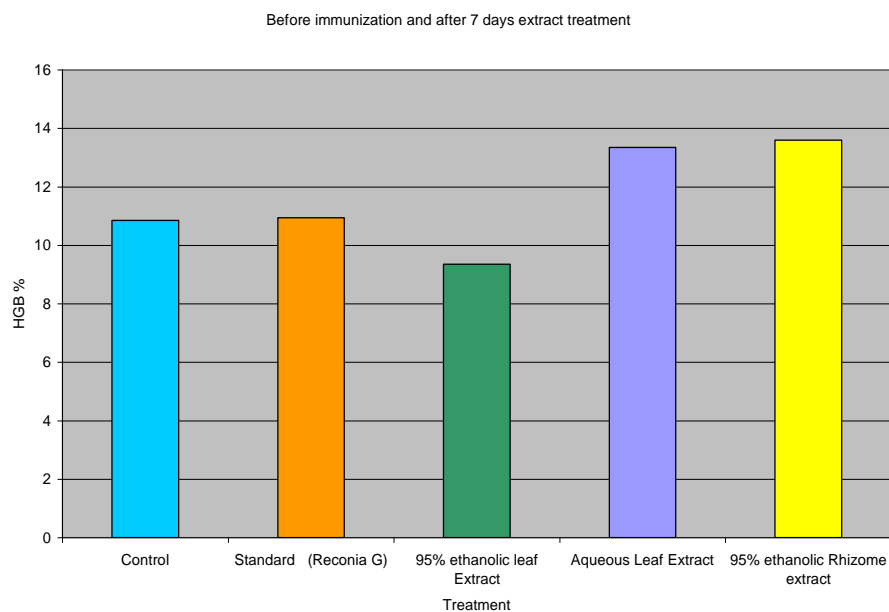
Before immunization and after 7 days extract treatment

Figure: 13 effect of different extract of *Curcuma Longa* on HGB count after 7days of extract treatment and before immunization

Before Immunization and after 7 days Extract treatment

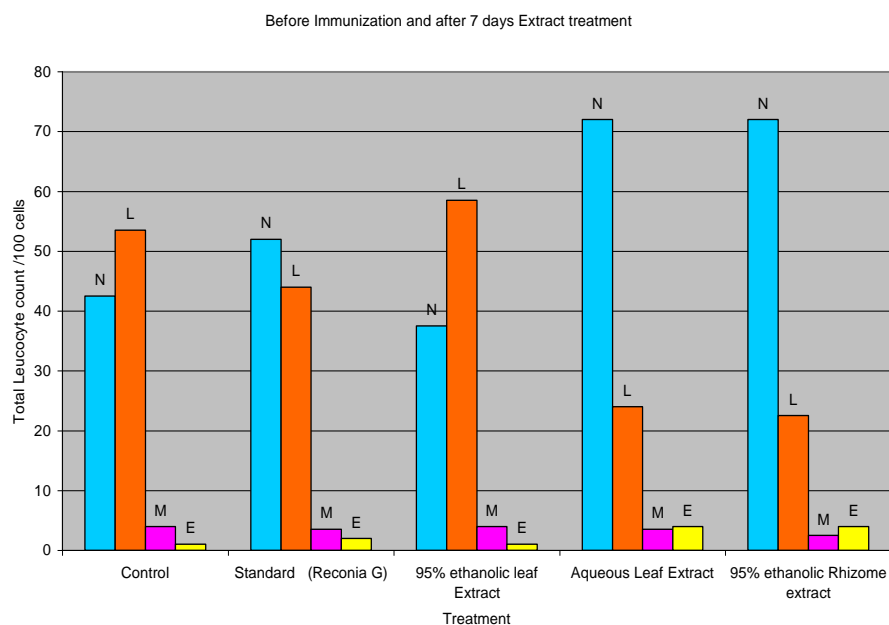**Figure: 14 Effect of different extract of *Curcuma Longa* on total Leucocyte count after 7days of extract treatment and before immunization**
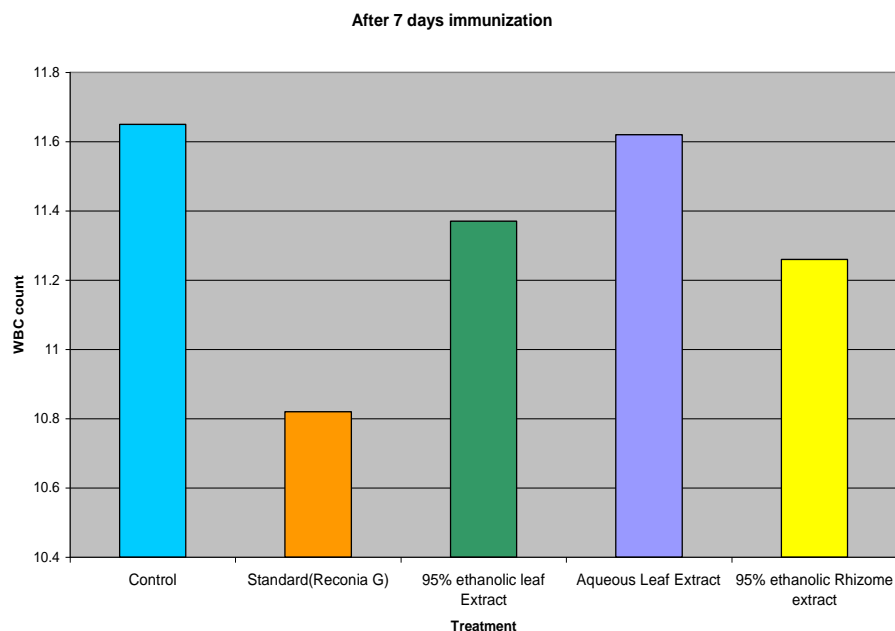
After 7 days immunization

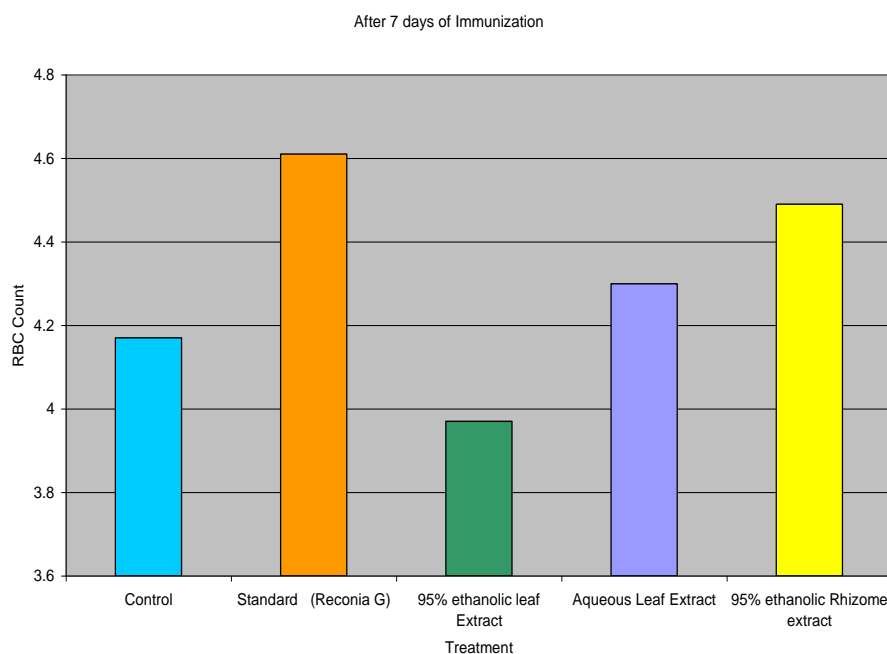**Figure: 15 Effect of different extract of *Curcuma Longa* on WBC count (10^3/cu mm) after 7days of Immunization**

After 7 days of Immunization

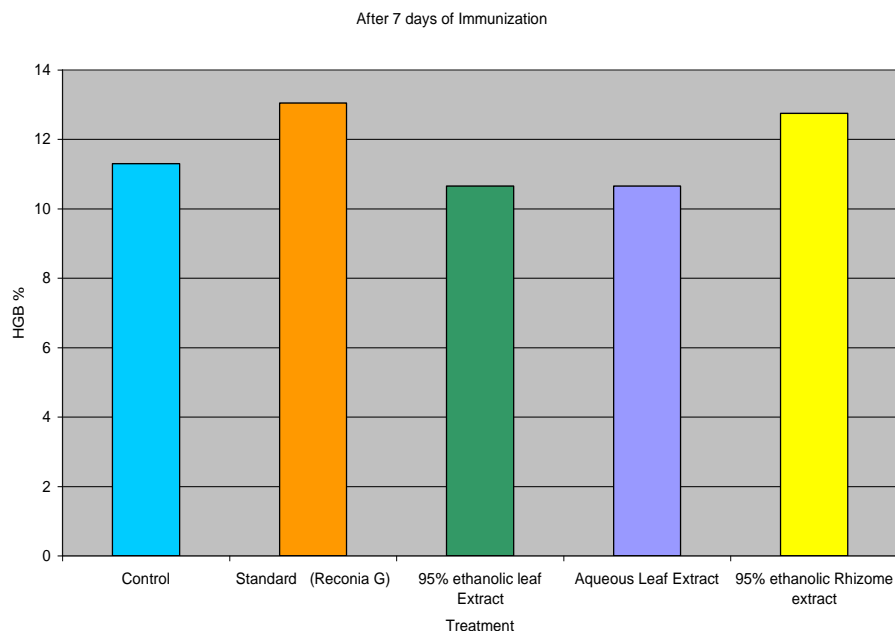**Figure: 16 Effect of different extract of *Curcuma Longa* on RBC count (10^6/cu mm) after 7days of Immunization**

After 7 days of Immunization

Figure: 17Effect of different extract of *Curcuma Longa* on HGB count after 7days of Immunization

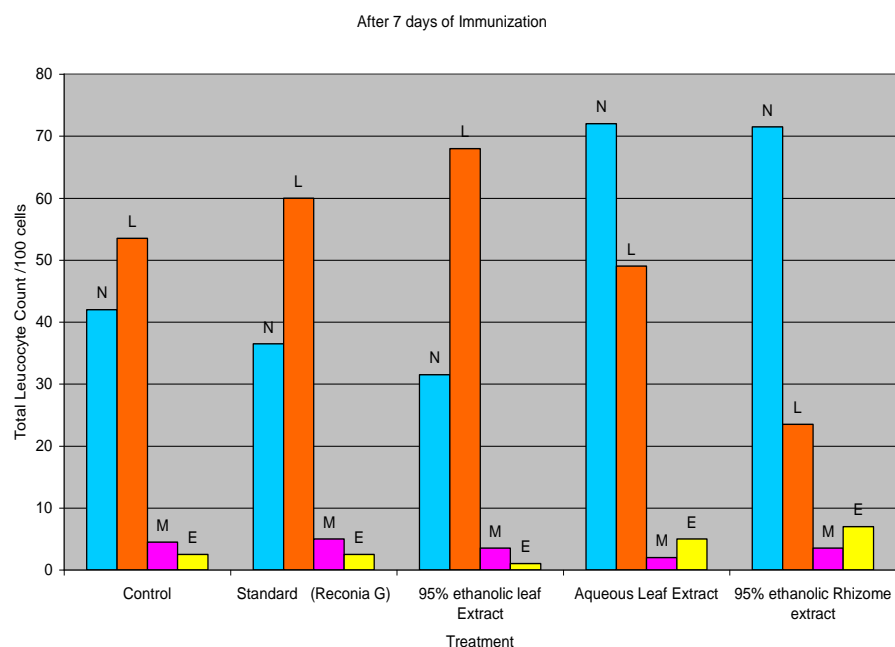After 7 days of Immunization

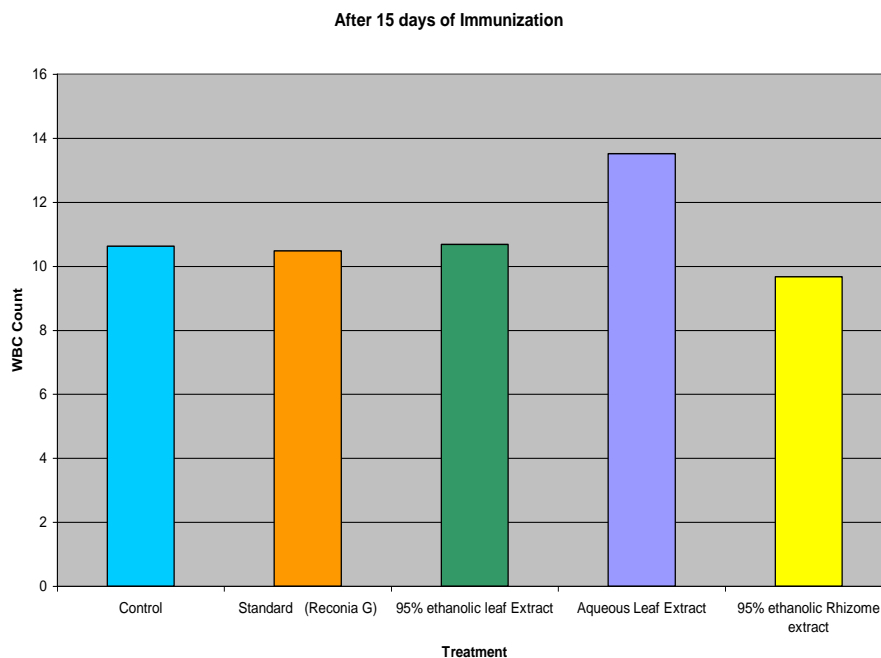**Figure18: effect of different extract of *Curcuma Longa* on total Leucocyte count after 7days of immunization**

After 15 days of Immunization

Figure19: Effect of different extract of *Curcuma Longa* on WBC count (10^3/cu mm) after 15days of immunization
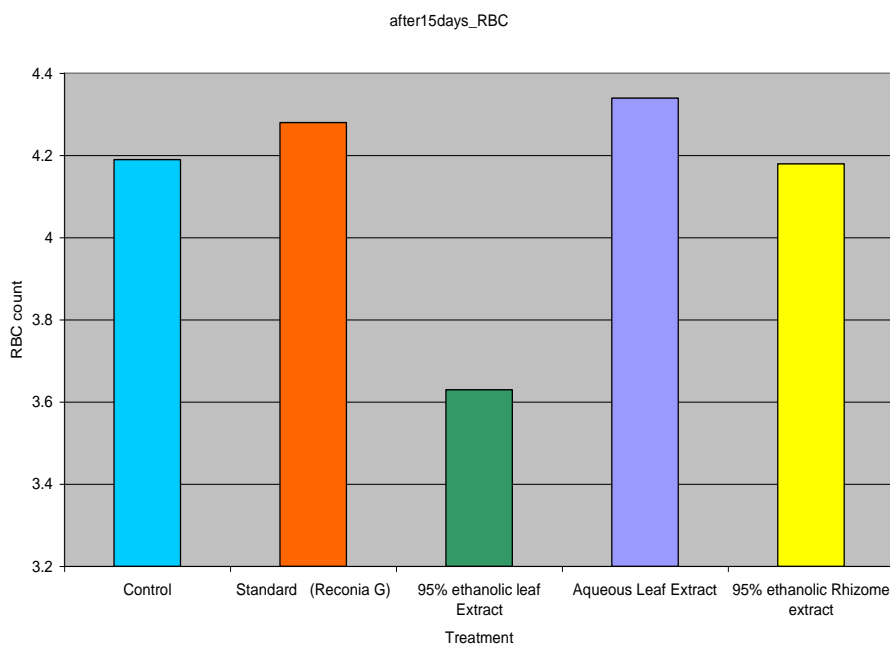
after15days_RBC

Figure20: Effect of different extract of *Curcuma Longa* on RBC (10^6/cu mm) count after 15days of immunization

After 15 days of Immunization

Figure21: Effect of different extract of *Curcuma Longa* on HGB count after 15days of immunization

After 15 days of Immunization

Figure22: Effect of different extract of *Curcuma Longa* on total leucocyte count after 15days of immunization
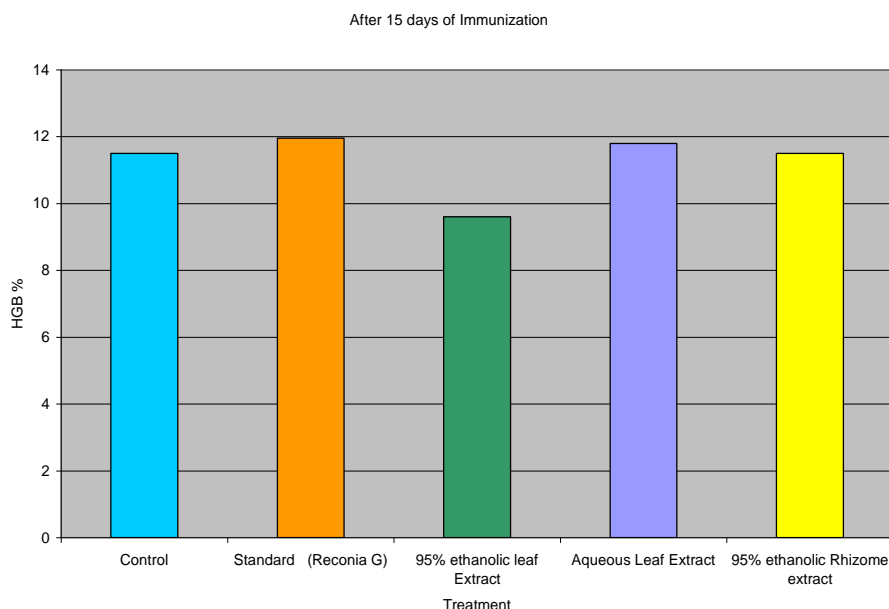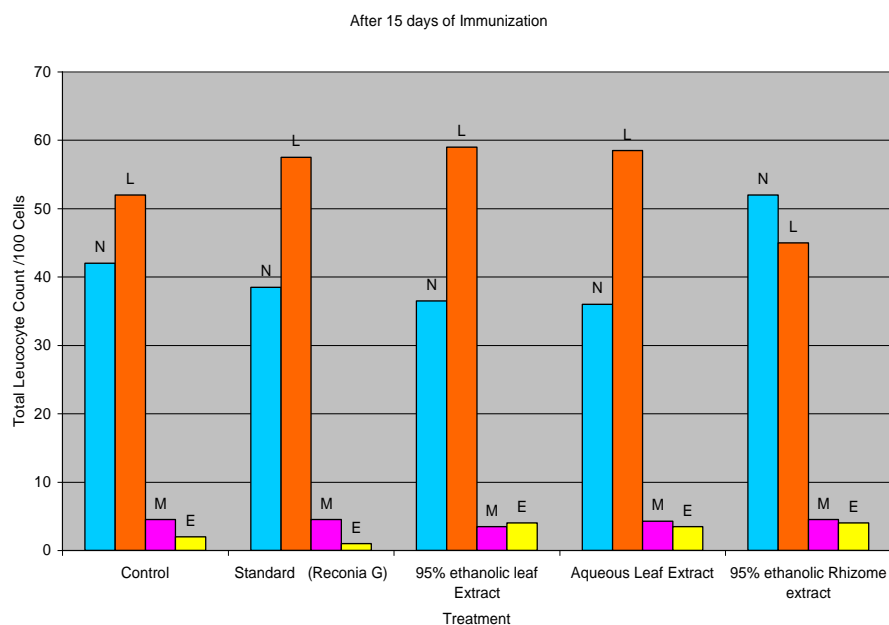
DISCUSSION AND CONCLUSION

Hematological activity

Hematological parameter, before immunization and after 7 day of drug treatment the WBC count of leaf extract are increases as compare to the standard, Aqueous leaf

extract and Rhizome Extract. After 7 days of immunization the neutrophil lymphocyte, RBC and HGB increased in the turmeric aqueous and leaf extract. After 15 day of immunization the WBC count increasing at the cost of RBC count, but HGB unaltered. This suggests the thought that drugs have immunostimulant effect. Such drugs are useful in crises to boost the immune system. *Curcuma Longa* aqueous leaf extract show the stimulant effect on RBC count.

Anti Bacterial Activity

In this study anti bacterial activity of three extract of *Curcuma Longa* i.e. 95% ethanolic leaf extract, Aqueous Leaf Extract, 95% ethanolic rhizome extract was monitored using disc diffusion method activity was determined by noting the zone of inhibition around the disc. *Curcuma Longa* is a well known indigenous herbal medicine having many biological* activities. It is well known spice, which is used as a dye, medicine and flavoring agent and exhibits a wide range of biological activities.

A five Gram negative Gram positive bacterial strain (5 strains of each) used. Antibacterial activity of three extract of *Curcuma Longa* was checked against these bacterial strains.

The study reveals that the 95% ethanolic leaf and rhizome extract at a concentration 12.5mg/ml were effective against Gram negative bacterial strain 95% ethanolic leaf extract show better antibacterial activity then the rhizome extract against the *Enterobacter aerogens* and *Streptobacillus monaliformus* (Gram negative) and *streptococcus epidermis* (Gram positive) bacterial strains and show no activity against three Gram positive bacteria (*Streptococcus pyrogens, Streptococcus aureus*, and *Streptococcus pneunoniae*) and Gram negative (*Escherichia coli*) (Gram negative)

Aqueous leaf extract exhibited no significant activity against the Gram negative and Gram positive bacterial strains.

Antibiotic Cefotaxime and Amikacin showed highest anti bacterial activity against Gram negative and Gram positive bacteria in the present set of studies. Present results reveal the potential medicinal use of turmeric leaf as antibacterial agents. Curcuma longa may

provide a valuable tool for the development of a therapeutic agent against both gram positive & negative microorganisms.

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