Nature has been a source of medicinal agents for thousands of years and an enormous number of modern drugs have been isolated from natural sources especially from plants; many of these isolations are based on the uses of the agents in traditional medicine. This plant based, traditional medicine system continues to play an essential role in health care, with about 80% of the world’s inhabitants relying mainly on traditional medicines for their primary health care (Owolabi et al., 2007).

The study of medicinal plants has attracted many researchers, owing to the useful applications of plants for the treatment of various diseases in humans and animals. To date, medicinal plants have been used in all cultures as a source of medicine for the treatment of various diseases including stomach complaints, malaria, depression, cancer and AIDS (Hoareau and Da Silva, 1999). Data has revealed that out of about 250000 flowering plants in the world more than 50000 are used for medicinal purposes (Schippmann et al., 2002).

Infectious diseases emanating from microorganisms such as bacteria, fungi, viruses and parasites are a major threat to public health care due to the growing resistance of many microorganisms to currently available antibiotics. The incidence of fungal infections has increased dramatically over the past few decades (Georgopapadakou and Walsh, 1994), mainly affecting immunocompromised or surgically treated patients as well as the young and old (Maertens et al., 2001). Immunocompromised patients with AIDS are commonly affected by fungal infections which cause morbidity and mortality.
With the rise in infections caused by various fungi and the development of resistance in fungal pathogens, it is important that novel antifungal agents be identified and developed (Alexander and Perfect, 1997).

Bacterial and fungal infections may be fairly easy to diagnose by traditional healers and community members, therefore there is more chance of finding a successful traditional remedy from plant material used in treatment of such infections. Previous studies have shown that plant species such as *Rhus javanica* L. have antifungal activity and has been used worldwide as a source of natural drugs (Ahn *et al*., 2005). Extracts from *Alpinia galanga*, *Curcuma zedoaria* and *Zingiber purpureum* were reported to have antifungal activity against a wide variety of human pathogenic fungi (Ficker *et al*., 2003) while some researchers have also found antifungal activity from extracts of *Aslepias curassavica*, *Bixa orellana*, *Eupotarium aschenbornianum* and *Galpinia galuca* (Garcia *et al*., 2003). Antimicrobial activity of plant parts has also been observed by various workers viz. Ripa *et al*, 2009; Chahal *et al*, 2010; Singh, 2011; Deepa *et al*, 2012; Sakunphueak *et al*., 2012; Dammal and Parthasarthi, 2013; Kang *et al*., 2013. These examples cited above reflect only a small representation of the work that has been carried out on the evaluation of plant extracts against microbial infectious agents.

Antimicrobials are compounds that at low concentrations exert an action against microorganisms and exhibit therapeutic toxicity towards them (Threlfall *et al*., 2006). These can be any substance of natural, synthetic or semi-synthetic origin that may be used to kill
microorganisms including bacteria, fungi and viruses (Yazaki, 2004). The antimicrobial activity of different plant extracts can be detected by observing the growth of various microorganisms that have been placed in contact with extracts of the plants. If the plant exhibits the growth of test organism and general toxic effects are not present, then the plant can potentially be used to combat diseases caused by the pathogens. The antimicrobial activities of plant extracts have formed the basis of many applications, including raw and processed food preservation, pharmaceuticals, alternative medicine and natural therapies (Lis-Balchin and Deans 1997).

There are several assays that can be used to determine antimicrobial activity in plant extracts, including agar diffusion, bioautography (direct, contact and overlay) and microplate assays (serial dilution assay). The agar diffusion assay is in general, only suitable for aqueous extracts and can also be used to test upto six extracts per petridish against a single microorganism. However, the diffusion method is not suitable for testing nonpolar samples or samples that do not easily diffuse into the agar. The bioautography assay is used to detect active compounds in a crude plant extracts (Cos et al., 2006).

Recently scientific interest in medicinal plants has burgeoned due to the increased efficiency of plant derived drugs and raising concern about the side effects of modern medicine. The rising prevalence of microorganism showing resistance to antibiotics has urged mankind to develop new antimicrobial compounds. Being non-toxic and easily affordable, there has been resurgence in the
consumption and demand for medicinal plants (Jayashree and Maneemegalai, 2008).

Knowing the ethnobotanical and pharmacological applications of the plant, the main objective of this research was to assess antimicrobial activity of leaf, stem and callus samples of *Cocculus hirsutus* against human pathogens. To screen for biological activity, the crude methanolic extract was prepared and tested against eight different microorganisms (four bacteria and four fungi). The aim of screening was to correlate and confirm the antimicrobial activity to the traditional uses of plants. This can be seen as a step in the search for primary health care products that are socially acceptable and scientifically valuable.

6A MATERIALS AND METHODS

(i) Collection of Plant material

Plant parts of *Cocculus hirsutus* were collected from Kulish Smriti Van, Jaipur and specimen was compared with the voucher specimen available at Herbarium of Department of Botany, University of Rajasthan, Jaipur. The fresh plant samples (*C. hirsutus*: leaf and stem) were collected and washed individually under running tap water to remove soil particles and other dirt. Furthermore, *in vitro* callus obtained on MS medium fortified with IAA (0.5 mg/l) was also taken for the present study.

(ii) Preparation of extract

The *in vivo* leaf and stem were dried in the laboratory at room temperature for 7 days while the callus was dried at 60°C for 2 days in
an oven. All dried samples were ground well into a fine powder in a mixer grinder. The powder was extracted by soxhlet extraction method using methanol as solvent. Later, the solvent was distilled under reduced pressure in a rotary vacuum evaporator until the extracts became dry. The crude evaporated plant extracts were dried at room temperature for 5-30 days. Then 50 mg of each crude plant extract was dissolved in 1 ml (1,000 microlitres) of the solvent to give a final concentration of crude extract in solvent of 50 mg/ml. Then, this extract was used for antimicrobial assay.

(iii) **Test Microorganisms**

Eight different strains of microorganisms were used in the screening process viz. (1) *Zymomonas mobilis* (MTCC 88), (2) *Staphylococcus epidermidis* (MTCC 3615), (3) *Staphylococcus aureus* (MTCC 3160), (4) *Micromonospora* sp. (MTCC 3296) and antifungal screening against (1) *Alternaria solani* (MTCC 2101), (2) *Fusarium culmorum* (MTCC 349), (3) *Phanerochaete chrysosporium* (MTCC 787), (4) *Penicillium chrysogenum* (MTCC 161) collected from the Microbial Type Culture Collection and Gene Bank (MTCC), Institute of Microbial Technology (IMTECH), Chandigarh, India. The bacteria were grown in nutrient broth (Himedia, M002) at 37°C and maintained on nutrient agar slants at 4°C and fungal cultures were grown and maintained on potato dextrose agar slants at 4°C.

(iv) **Antimicrobial activity**

Antimicrobial assay of the crude extracts was performed against eight tested pathogenic strains by Agar-well diffusion method. The
bacterial strains were grown on nutrient agar medium (agar 15 gm, beef extract 3 gm, sodium chloride 5 gm and peptone 5 gm, in one liter distilled water) at 37°C for 18 h and were suspended in a saline solution (0.85% NaCl) and adjusted to a turbidity of 0.5 Mac Farland standards (108 CFU/ml). The suspension was used to inoculate 90 mm diameter petriplates. Wells (6 mm diameter) were punched in the agar and filled with the test samples (crude methanol extracts of flower and leaf samples of plant) to get different concentrations viz. 15, 25, 35, 45, 55 µl of the extract. Ampicillin was used as a standard for antibacterial assay and Flucanazol for antifungal assay. Plates were incubated at 37±2°C for 24 hours. Antimicrobial activities were evaluated by measuring inhibition zone diameters and the activity index was calculated for each of these. The experiments were conducted in triplicate. The same method was followed for testing antifungal activity using potato dextrose agar medium.

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\text{Activity index} = \frac{\text{Inhibition zone of the sample}}{\text{Inhibition zone of the standard}}
\]

(v) **Determination of minimum inhibitory concentration (MIC)**

Minimum inhibitory concentration of various extracts against tested microorganisms was determined by broth dilution method (Basri and Fan, 2005). For broth dilution, 1ml of standardized suspension of a strain (10^6 cfu/ ml) was added to each tube containing extracts at various concentrations in nutrient broth medium. The tubes were incubated at 37°C for 24 hours (for bacterial strains) and 25°C for 48 hours (for fungal strains) and observed for visible growth after vortexing them gently. The minimum inhibitory concentration (MIC)
is taken as the lowest concentration of the extracts at which there is turbidity after incubation. The highest dilution of a plant extract that still retains an inhibitory effect against the growth of microorganisms is known as MIC.

6B OBSERVATIONS AND RESULTS

The antimicrobial activity of methanolic extract of leaf, stem (in vivo) and callus (in vitro) against bacteria and fungi examined in the present study and its potency was quantitatively assessed by the presence or absence of inhibition zones and zone diameters.

The results of the antimicrobial activity are presented in Table-32 [Plate-42&43]. Antimicrobial screening of methanolic extract from leaf, stem and callus of Cocculus hirsutus revealed that the methanolic extract of callus showed better antimicrobial activity against microorganisms as compared to methanolic extract of leaf and stem part. Maximum zone of inhibition was obtained with methanolic extract of callus as compared with leaf and stem.

Maximum zone of inhibition was observed in the callus extracts against Staphylococcus epidermidis (3.9 ± 0.56 mm) [Table-32, Plate-42, Fig. C] amongst the bacteria species and against Phanerochaete chrysosporium (2.5 ± 0.63 mm) [Table-32, Plate-43, Fig. B] amongst the fungal species. Methanolic extracts of leaf and stem showed varied activity with different strain of bacteria and fungi. Methanolic extract of leaf and stem showed maximum inhibition zone against Micromonospora sp. bacterial strain. Zone of inhibition obtained with leaf extract was 2.3 ± 0.78 mm and stem extract was 2.5 ± 0.48 mm while in case of fungal strain maximum inhibition zone
was observed against *Fusarium culmorum*. Zone of inhibition obtained with leaf extract was 1.9 ± 0.73 mm and stem extract was 2.1± 0.47 mm.

The MIC method was used to further investigate extracts that showed broad spectrum activity against microorganisms. The highest dilution of a plant extract that still retained an inhibitory effect against the growth of microorganisms (absence of zone of inhibition) was reported as the MIC. In this study, methanolic extract of callus sample showed highest promising MIC of 61.1 µgml⁻¹ in *Staphylococcus epidermidis* (Table-33). Results of MIC value of different plant parts are summerised in Table-33. The minimum inhibitory concentration (MIC) of the stem extract for different micro-organisms ranged between 17.8 to 45.5 µgml⁻¹, while that of the leaf extract ranged between 20 to 59.3 µgml⁻¹ ((Table-33)). The MIC of Ampicillin and Flucanazol control ranged between 19 to 33.21 µgml⁻¹.

6C CONCLUSIONS

- From the results obtained in this study, it is evident that the methanolic extract of different samples of *Cocculus hirsutus* is effective against all the tested pathogens.

- These results reveal that the methanolic extract of callus of *Cocculus hirsutus* could be a potential source of traditional medicine for infections caused by tested micro-organisms.

- Further studies are necessary to elucidate the exact bioactive compound which is responsible for the destined antimicrobial action.