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PHYTOCHEMICAL SCREENING AND ANTIBACTERIAL ACTIVITY OF *CROCUS SATIVUS* PLANT

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INTRODUCTION

Nature has been a source of medicinal agents since times immemorial. The importance of herbs in the management of human ailments cannot be over emphasized. It is clear that the plant kingdom harbors an inexhaustible source of active ingredients in the management of many intractable diseases. Furthermore, the active components of herbal remedies have the advantage of being with many other substances that appear to be inactive however, these complementary components give the plant as a whole safety and efficiency much superior to that of its isolated and pure active components.

Recently, multiple drug resistance has developed due to indiscriminate use of commercial antimicrobial drugs commonly use as treatment of infection diseases making it a global growing problem. isolation of microbial agents less susceptible to research and recovery of increasing resistant isolation during antibacterial therapy is rising throughout the world, which high light research principles. Natural products of higher plants may give a new source of antimicrobial agents with possibly novel mechanism therapeutic potential to heal many infection diseases (Parekh and Chandra 2007).

Since the beginning of time, people have used plants to treat common illnesses, and many traditional medicines are still used today to treat a variety of illnesses on a regular basis.[1] The use of traditional medicines is still a necessity for about 60% of the world's population.[2] The use of various portions of various medicinal plants to treat various ailments has been popular since ancient times in India, where thousands of species are known to have medicinal benefits.[3] The use of medicinal plants, which have undergone testing for biological, antibacterial, and hypoglycemic activities and are recognized as potentially safe medications,

is significant in contemporary medicine.[4,5] The origin of even the most synthetic medications is generally recognized to come from plant materials.[6] Due to the rising effectiveness of plant-derived medications and growing concern about the negative side effects of contemporary treatment, there has been a recent explosion in scientific interest in medicinal plants. Due to the ongoing growth of drug-resistant organisms and the adaptations made by microbial pathogens to routinely used antimicrobials, the effectiveness of present antimicrobial medications has been decreased. As a result, one of the main sources of commercial pharmaceuticals continues to be the quest for new medications in plants. Further research into plant-based antimicrobials is necessary since they still represent a sizable untapped supply of medications despite their huge therapeutic potential and success in treating infectious diseases.[7] Higher plants are a possible source of novel antibiotic prototypes, according to the screening of plant extracts and their products for antimicrobial activity.[8] In the early stages of screening procedures, choosing crude plant extracts rather than purified chemicals may be more successful.[9] Numerous researchers have already examined this screening of different plant extracts.[10,11] The bulk of plant species have not yet been assessed, despite the fact that hundreds of plant species have been investigated for antibacterial characteristics.[12]

Herbal remedies represent one of the most important fields of traditional medicines in Rajasthan especially in tribal areas. Thus, phytotherapy is practiced by a large proportion of tribals of Rajasthan for treatment of several physical, physiological and social ailments. To promote the proper uses of herbal medicines and to determine their potential as source of new drugs, it is important to study medicinal plants, which have folklore reputation in more intensified way.[1-4] Within the recent years, infection has increased largely and antibiotic resistance becomes an ever-increasing therapeutic problem.[5] Natural products of higher plants may give a new source of antimicrobial agents with possibly novel mechanism of action.[6-9] In recent years, multiple drug resistance has developed due to indiscriminate use of existing antimicrobial drugs in treatment of infectious diseases[10] Meyer et.al 1996). In fact, there are several medicinal plants all over the world, including India, which are being used traditionally for the prevention and treatment of cancer. However, only few medicinal plants have attracted the interest of scientists to investigate the remedy for neoplasm (tumor or cancer).

MATERIAL AND METHODS

Plant collection &identification: Thread Like part of flower of *Crocus Sativus* were collected from Pampore J&K. The plant was identified by Dr. Kewal Assistant Professor of botany GCW Udhampur J&K.

Extraction of plant material: Plant extract were prepared by cold percolation method. The air-dried and powered plant material (5gm) each was soaked in 50 ml ethanol and kept for 48 hours with intermittent shaking. The plant extract were filtered through whatman no. 1 filter paper. The filtrated were dried until a constant dry weight of each extract was obtained. The crude extract were dissolved in 30% dimethylsulphoxide and further diluted to obtain 250,200,150,100 & 50mg/ml concentrations. Each extract was dissolved in 1 ml Dimethyl sulfoxide (DMSO) and 20 µl of each sample is taken for explement.

Antimicrobial screening: The methanolic extract of *Crocus Sativus* screened against five microorganisms. The test organisms were *E. coli., S.aureus, P.aeruginosa, B.subtils, S.typhi* obtained from the department of microbiology Gandhi medical college Jammu. The modified well diffusion method (Bauer AW et al 1966) was used to screen the antimicrobial activity. Sterile filter paper discs of 6 mm diameter impregnated with 20 µl extract solution (equivalent to 4mg of the dried extract) and after evaporation placed on the surface of the inoculated agar plate and the compound was allowed to diffuse for 5 minutes, and plates were kept for incubation at 37°C for 24 hours. At the end of incubation, inhibition zones around the disc were measured with transparent ruler in mm. The same procedure was followed for the fungus also. The study was performed in triplicate. Amikacin was used as control against all pathogens.

RESULT AND DISCUSSION: Table 1 displays the antibacterial screening results for the test isolates using various extract concentrations. The results indicate that some bacteria' growth inhibition zones are expanding. The growth of S. typhi was not inhibited by the extract at any of the supplied concentrations. The extract's 250 mg/ml concentration against S. aureus produced the maximum zone of growth inhibition, measuring 13.5 mm in diameter. Only concentrations of 200 and 250 mg/ml had an impact on B. subtilis, E. coli, and P. aeruginosa. The extract was used against B.subtilis at a concentration of 200 mg/ml, resulting in the lowest zone of growth inhibition, which measured 5.6 mm. The minimum inhibitory concentration of the extract on the test isolates are shown in table 2. The lowest minimum inhibitory concentration (MIC) was produsd against S.aureus with a concentration of

22.55mg/ml while the highest MICwas against B.subtils with a concentration of 74.61mg/ml. The extract was found to contain tannins, alkaloids, flavanoids, cardiac glycosides. Saponins and cyanogenic glycosides were not present in this plant. The results obtained indicated that the ethnolic extract of the E.hirta inhibited the growth of the test isolates except S.typhi. This therefore shows that the extract contains substance that can inhibit the growth of some microorganisms. Other workers have also shown that extract of some plants inhibited the growth of various microorganisms at different concentrations (Akujobi et al, 2004, Esimone et al 1998, Nwezeet al 2004, Ntiejumokwu and alemika 1991, Osadebe and ukwueze2004). The observed antibacterial effects on the isolates is belived to be due to the presence of alkaloids, tannins and flavanoids which have been shown to posses antibacterial properties(cown, 1999; Draughon, 2004). Some workers have also attributed their observed antimicrobial effects of plant extracts to the presence of these secondary metabolites(Nweze et al, 2004). Its use in conventional medicine is supported by the antibacterial activities that have been observed. Traditional uses of the plant's extracts include treating sores and wounds, treating ear infections, and controlling diarrhea and dysentery (Kokwaro, 1993; lgoli et al. 2005). The extract's wide zones of inhibition against S. aureus and P. aeruginosa supported its use by traditional healers to treat ulcers, bores, and open wounds. According to Breude (1982), S. aureus and P. aeruginosa have been linked to cases of boils, ulcers, and wounds. Additionally, its application in the management of diarrhea and dysentery is justified by the mild growth inhibition against Ecoli. According to Adams and Moss (1999), E. coli is frequently to blame for human cases of traveler's diarrhea and other diarrheagenic diseases. The extract's low MIC against S. aureus is extremely important for the treatment of infections brought on by these organisms, especially given that they regularly become resistant to established antibiotics (Singleton, 1999). Their use will also lower the price of receiving medical care. Given that E. coli is a common cause of diarrhea in developing nations, the extract's relatively high zone of inhibition against E. coli is especially significant. The fact that Salmonella typhi has a mechanism for detoxifying the extract's active ingredients may explain why the extract is unable to stop it. It is known that some bacteria have the ability to transform chemicals that stop their growth into non-toxic molecules. As an illustration, the bacteria S. aureus develops the enzyme penicillinase, which changes the antibiotic penicillin into penicillinoic acid, which is no longer growth-inhibitory. (Singleton 1999).

Table 1: Antibacterial screening of the different concentrations of crude methanolic extract of *Crocus Sativus.*

Concentration of Extract (mg/ml)	Zone of inhibition (mm)				
	E.coli	***S.aureus***	***P.aeruginosa***	***B.subtilis***	***S.typhi***
250	11.9	13.5	12.1	7.3	NI
200	8.9	12.9	12.3	6.6	NI
150	8.0	12.5	6.2	6.6	NI
100	6.9	10.6	6.5	NI	NI
50	3.9	7.8	NI	6.6	NI

Values are means.NI=No inhibition.

Table 2: Minimum Inhibitory concentration of the methanolic extract of *Crocus Sativus* against test isolates.

Plant	***E.coli***	***S.aureus***	***P.aeruginosa***	***B.subtilis***	***S.typhi***
Crocus Sativus	68.09	25.55	59.64	79.69	Nil

Table 3: Preliminary phytochemical screening of extract of *Crocus Sativus.*

Plant	Saponons	Tannins	Flavonoids	Alkaloids	Cardiacglycosides
Crocus Sativus	-	+	+	+	+

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