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**Antibacterial analysis of crude extracts from the leaves of *Tagetes erecta* and *Cannabis sativa***Das B<sup>1</sup>, Mishra P C<sup>2</sup>

1- Institute of Mineral and Material Technology, Bhubaneswar, Odisha, India.

2- MITS School of Biotechnology, Bhubaneswar, Odisha, India

banani.liza@gmail.com

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**ABSTRACT**

Leaves of *Tagetes erecta* and *cannabis sativa* traditionally practiced in the treatments of boils, dysentery, indigestion and various skin infections. The aim of this study was on the one hand to extract crude alkaloid from the leaves of *Tagetes* and *Cannabis* plants and on the other hand to evaluate the in vitro antibacterial activities of the crude extract against bacterial strains representative of skin, mouth and ear microflora and also against  $\beta$ -strain of *E.coli* bacteria responsible for urinary tract infection in human beings by disc diffusion method. The active ingredient of *Tagetes erecta* and *Cannabis sativa* was identified as alkaloid by Mayer's and Dragendorff's test. The antimicrobial activities were compared with two standard antibiotics Chloramphenicol and Tetracycline. However, this study revealed maximum growth inhibition and effectiveness was remarkably observed in the extracts of *Cannabis sativa* and then in *Tagetes erecta*. These results indicate that leaves have a potential broad spectrum antibacterial activity.

**Keywords:** *Tagetes erecta*, *Cannabis sativa*, Antibacterial activity, Disc diffusion, Active compound.

**1. Introduction**

Alkaloids are natural organic substances that are predominantly found in plants. Alkaloids can occur in all part of the plant but frequently depending on the plant species, they accumulate only in particular organs such as in barks, roots, leaves and fruits; where at the same time other organs are alkaloid free. Many synthetic and semi synthetic drugs are structural modification of the alkaloids which were designed to enhance or change the primary effect of the drug and reduce unwanted side effects. The primary benefits of using plant derived alkaloids as medicines are that they are relatively safer than synthetic alternatives, offering profound therapeutic benefits and more affordable treatment. Where as synthetic antibiotics have many adverse effects such as diarrhea resulting from disruption of the species composition in the intestinal flora.

Antibiotic can also affect the vaginal flora and may lead to overgrowth of yeast species in the vulvo-vaginal area. Additional side effects can result from interaction with other drugs. Natural antibiotics overcome the problems inherent with synthetic antibiotics. When used properly, natural antibiotics can boost the body's ability to produce antibiotics for which bacteria as well as viruses and fungi cannot develop immunity. Preparations of plants containing alkaloids and their extracts and later pure alkaloids have long been used as psychoactive substances. Now a day's many alkaloids are also used as pesticides in agricultural fields.

In this study we tried to extract crude alkaloids from 2 different plants such as *Tagetes erecta* and *cannabis sativa* to investigate the antimicrobial activity of the extract against some pathogenic bacteria. *Tagetes erecta* is a member of composite family, the blossoms are actually clusters of flowers, the plant reaches a heights between 50-100 c.m. There are 20-30 species are found in India. (Boland et al., 1991). The plant is cultivating for medicinal, ceremonial and decorative purposes. This species are scientifically investigated for their potential benefits to health. The whole herb is anthelmintic, aromatic, digestive, diuretic, sedative and stomachic. It is used internally in the treatment of indigestion, colic, severe constipation, cough and dysentery. Externally it is used to treat sores, ulcers, eczema, sore eyes and rheumatism (Kim et al., 2005). *Cannabis sativa* belongs to the family cannabaceae is most popular herb found in India as well as across the world (Kim et al., 2005). It is well known for its fiber known as hemp which has prominent psychological and physiological effects. Its seed chiefly used as caged-bird feed, is valuable source of protein. From ancient times tinctures, teas and ointments have also been in common preparations.

## 2. Methodology

Fresh leaves of desired plants were collected, washed and sun dried for 72 hours. The dried leaves were smashed mechanically and the pulverized leaves were sieved to obtain only the leaves without the midribs. To 500gm of powder 1 liter of petroleum ether was added and stirred using mechanical force for around 2 hours, after stirring the mixture was left overnight to soak in petroleum ether. Then this mixture was filtered with the help of muslin cloth. The treated leaves were then mixed with 1 liter of methanol and incubated for 72 hours. After the incubation period the mixture was filtered to obtain the crude plant residue. The crude plant residue was again mixed with 1 liter of distilled water and acidified with the help of concentrated sulphuric acid till the solution attains pH 2. The acidified crude plant mixture is subjected to steam distillation to collect the desired part containing a mixture of water and salt of alkaloids (Upadhyay A., 2003).

The product that was obtained was about 400ml, a small fraction about 20ml was taken in a beaker and to it equal volume of 0.1N NaOH solution was added and equal volume of ether was added. The NaOH removes the acid group from the salt of alkaloid leaving the alkaloid to dissolve in ether. Now the mixture was separated with the help of separating funnel.

### 2.1 Testing for the presence of alkaloids

#### Mayer's Test

Mercuric chloride ( $HgCl_2$ ) - 1.358gm in 60ml water

Potassium Iodide (KI) – 0.5gm in 10ml water

Both the solution was added and the volume was made up to 100ml with Mayer's Reagent it gives a cream colour precipitation which is positive result for the presence of alkaloids (Ammon et al., 1985).

#### Dragendorff's Test

Bismuth Nitrate - 0.85gm in 10ml acetic acid and 40ml water.

Potassium Iodide (KI) - 8gm in 20ml water.

Dragendorff's Reagent - 5ml of Bismuth Nitrate + 5ml of KI + 20ml acetic acid then the final volume was made upto 100ml with water.

With Dragendorff's reagent it gives an orange precipitate which is a positive result for the presence of alkaloids (Ammon et al., 1985).

## 2.2 Antimicrobial activity test

Samples were collected from mouth, skin and ear and cultured in separate plates with Nutrient agar media and incubated for 24 hours in 37°C. After growth of micro-organism they were identified with the help of gram's staining. The micro-organisms were again cultured by spread plate method to obtain the pure culture (Prescott et al., 4th edition).

Pure culture of  $\beta$ -strain of *E.coli* isolated from urine sample of patient suffering in urinary tract infection was also taken and cultured in separate petridish. At the center of each plate a circular paper strip dipped in crude alkaloid is placed. Two reference plate of each sample is provided with 2 antibiotic strips such as chloramphenicol and Tetracycline. Plates were now incubated for 24 hours at 37°C (Ryan et al., 2004).

## 3. Result

**Table 1:** Inhibition zone pattern of bacterial samples in selected alkaloids with reference to synthetic antibiotics

| Bacterial sample            | Inhibition Zone Diameter in cm |                       |                 |              |
|-----------------------------|--------------------------------|-----------------------|-----------------|--------------|
|                             | <i>Cannabis sativa</i>         | <i>Tagetes erecta</i> | chloramphenicol | Tetracycline |
| E.coil                      | 2.5                            | 2.2                   | 2.1             | 1.9          |
| Mouth microflora (gram +ve) | 2.2                            | 1.9                   | 1.9             | 1.6          |
| Skin microflora (gram +ve)  | 2.1                            | 2.0                   | 2.4             | 2.2          |
| Ear microflora (gram +ve)   | 1.7                            | 1.6                   | 1.7             | 1.6          |

## 4. Discussion

*E.coli* is a gram- negative pathogenic bacterium. The  $\beta$ -strain of *E.coli* is responsible for urinary tract infection in human beings. The crude alkaloids extracted from *Cannabis sativa* and *Tagetes erecta* shows zone of inhibition of 2.5cm and 2.2cm respectively as compared to chloramphenicol and tetracycline. Thus we can conclude that these crude alkaloids may be used as a drug to treat such urinary tract infection or may be used in combination with antibiotics.

We isolate gram-positive bacteria from mouth sample. The mouth microflora includes gram positive bacteria like *streptococci* and *staphylococci* species. Streptococcus species like *S.mutans*, *S.sorbrinus*, and *S.sanguis* are widely present in mouth. These species are known to be causing tooth decay by producing lactic acid. Streptococcus species are responsible for

strep throat, scarlet fever, rheumatic fever, toxic shock syndrome. Crude alkaloid from *cannabis* and *Tagetes* shows a significant zone of inhibition of 2.2cm and 1.9cm respectively as compared with chloramphenicol and tetracycline showing zone of inhibition of 1.9cm and 1.6cm respectively. Thus we may conclude that this crude alkaloid may be effective in treating the bacterial infection described as earlier or may be used in combination with the antibiotics to treat infection more effectively.

Skin of human being act as a heaven for gram-positive bacteria like *Streptococcus* and *staphylococcus* species. *Staphylococcus aureus* is one of the most frequently found gram-positive bacteria in skin microflora. They cause minor skin infection like pimple, boils, scalded skin syndrome, abscesses, cellulitis etc. They may cause life threatening diseases such as pneumonia, meningitis, septicaemia etc. Streptococcus skin infection includes cellulitis, impetigo, perianal and vulval dermatitis etc. It was noted that the inhibition zones of the crude alkaloid differ from the inhibition zones of standard antibiotics. Here chloramphenicol and tetracycline are showing a zone of inhibition of 2.1cm and 2.2cm respectively where the crude alkaloid of cannabis and *Tagetes* shows a zone of inhibition of 1.8cm and 2.0cm respectively.

Ear is home to various harmful gram-positive bacteria like *Streptococcus pneumoniae* which causes pneumonia. In the result we have seen that crude alkaloid described from *Cannabis sativa* and *Tagetes erecta* is showing a zone of inhibition of 1.7cm and 1.6cm, while the antibiotics like chloramphenicol and tetracycline showing zone of inhibition of 1.7cm and 1.6cm respectively.

## 5. Conclusion

Extract of *Cannabis sativa* and *Tagetes erecta* in this study demonstrated a broad-spectrum of activity against both gram-positive and gram-negative bacteria. The broad spectrum antibacterial activities of the plant extract is due to the identified alkaloids. So the crude alkaloid can be used as a potential drug separately or in combination with other antibiotics.

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