AN EVALUATION INVITRO ANTIOXIDANT ACTIVITY OF HEMEDESMUS INDICUS ROOT EXTRACT BY DPPH METHOD

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Abstract

For Hemedesmus indicus, in vitro antibacterial activity was assessed using root extract. In addition, the antioxidant potential of the root extract was evaluated using the DPPH method, nitric oxide scavenging activity, and reducing power assay method. Furthermore, the anti-inflammatory activity was determined through protein denaturation and HRBC membrane stabilization methods. The in vivo analgesic and anti-inflammatory effects of Hemedesmus indicus root extract were examined using various animal models, including the tail flick method, hot plate method, and acetic acid-induced writhing responses method.

Similarly, for Justicia Betonica, preliminary phytochemical tests were performed, followed by in vitro antibacterial activity evaluation of the root extract. The antioxidant activity of the extract was assessed using the DPPH method, nitric oxide scavenging activity, and reducing power assay method. The anti-inflammatory potential was investigated through protein denaturation and HRBC membrane stabilization methods. The in vivo analgesic and anti-inflammatory effects of Justicia Betonica root extract were examined using the tail flick method, hot plate method, and acetic acid-induced writhing responses method.

INTRODUCTION

Medicinal plants have been a vital source of both curative and preventive medical therapy for human beings ailments (Madsen et al., 2009). It is estimated almost 85% of the world 's total population depends upon traditional ayurvedic medicinal products for its health care needs especially in third world countries.Traditional and folkloric medicines are usually economically cheap and produce significant therapeutic effects for the treatment of various diseases, not only rural but also urban dwellers nowadays prefer traditional medicines because of their close proximity to traditional healers. Ethnobotonical investigations are considered to be very vital in preserving traditional medicines through proper documentation of medicinal plants which assist its sustainability. Modern pharmacopeias do contain more than 20% of the drugs obtained from plants and other natural sources which are synthesized based on isolated compounds from plants origin. The trend of using these herbal medicines are increasing in both developed and developing countries due to their less side effects and no alternative source to the poor patients for their health care (Machel et al., 2003).

Traditionally applied medicinal plants are of great importance. In case of effectiveness and safety, the authorization often relies on long-term medicinal use. However, the phamacovigilance data of medicinal herbs reveal a need for continuous re-evaluation (Sanjay et.al., 2007).

Modern approaches

Modern approaches of phytochemistry, pharmacology and toxicology might lead to new breakthroughs in drug research.

The better understanding of traditional application and processing of medicinal herbs resulted in the discovery of novel mechanisms of action and new active constituents of these plants (Khan et.al., 2003). The chemical analysis of raw plant materials and processed drugs made it possible to identify their biological active compounds and their change during processing

A worldwide revival of interest in herbal drugs has been there due to the following reasons.

- High cost involved in synthetic drugs development.
- In the manufacture of synthetic drugs non-renewable raw materials are used.
- Industrial emissions during the production of synthetic drugs.
- Medicinal plants can be considered as renewable sources.
- The cultivation and processing cause very less pollution when compared to the synthetic drugs.

The plants importance is very well known (Joshi et. al 2004). Drugs from the plants are available very easily, economical, high safety, very effective with few adverse effects. (Vinod et.al., 2006) The plants which were used for treatment several thousand years earlier constitute the most obvious choice of monitoring the present research for therapeutically significant novel drugs like antineoplastic agents (Dewick, P.M.1996), antimicrobial drugs, antihepatotoxic compounds.Traditional medicines are used by the majority of people in the developed countries which mainly consists of compounds that are obtained from the medicinal plants.However detailed investigation has to be carried out on those plants for the better understanding of their properties, safety and efficacy (Christ et. al. 1998). As the medicinal plants mainly contain organic compounds and several bioactive substances which includes tannins, alkaloids, terpinoids and steroids which exhibit various important physiological actions. From ancient times the phytomedicines mainly consist of the plant products either leaves, flowers, roots, fruits, seeds and barks, are mainly obtained. (Kumar et al., 2003) (Khan et al., 2006)

Medicinal plants have been used in healthcare since time immemorial. Studies have been carried out globally to verify their efficacy and some of the findings have led to the production of plant-based medicines. The global market value of medicinal plant products exceeds $100 billion per annum. The place of medicinal plants in preventing common diseases is further examined under the five core principles of the Primary Health Care (PHC) approach (Jain et al., 2010) (Babu et al., 2004). Medicinal plants play vital roles in disease prevention and their promotion and use fit into all existing prevention strategies. However, conscious efforts need to be made to properly identify, recognize and position medicinal plants in the design and implementation of these strategies (Clark et al., 2007). These approaches present interesting and emerging perspectives in the field of medicinal plants. Recommendations are proposed for strategising the future role and place for medicinal plants in disease prevention. A number of plants have been used in traditional medicine for many years. Some do seem to work although there may not be sufficient scientific data (double-blind trials, for example) to

confirm their efficacy. Such plants should qualify as medicinal plants (Williams et al., 2001). The term 'crude drugs of natural or biological origin' is used by pharmacists and pharmacologists to describe whole plants or parts of plants which have medicinal properties.

a. plants or plant parts used medicinally in galenical preparations (e.g. decoctions, infusions, etc.) e.g. Cascara bark;

b. plants used for extraction of pure substances either for direct medicinal use or for the hemi-synthesis of medicinal compounds (e.g. hemi-synthesis of sex hormones from diosgenin obtained from Dioscorea yams);

c. food, spice, and perfumery plants used medicinally, e.g. ginger;

d. microscopic plants, e.g. fungi, actinomycetes, used for isolation of drugs, especially antibiotics. Examples are ergot (Claviceps purpurea growing on rye) or Streptomyces griseus; and

e. fibre plants, e.g. cotton, flax, jute, used for the preparation of surgical dressings.

The growing importance of medicinal plants can be appreciated from the economic stand point when the following facts are considered:

- Global trade in herbs is over USD 100 Billion per annum
- India and China's medicinal plant trade is about two to five billion US dollars annually
- In Germany, it is over one billion US dollars annually
- Rose Periwinkle which is endemic to Madagascar fetches US$100 million per annum
- China trades in 7,000 species and 700,000 tons of medicinal plants per annum
- India trades in 7,000 species of medicinal plants
- Morocco exports 58.7 tons of medicinal plants annually
- In the last 5 years, sales of medicinal plants doubled in China, tripled in India and grew by 25% in Europe.

Preventive strategies

- Health promotion, disease prevention and chronic disease management are proactive approaches to health care that stresses prevention at different points along the health care continuum. Health promotion and disease prevention strategies focus on keeping people well and preventing diseases from occurring (Gibbs et al., 2009)(Venkata et., al 2008). These strategies are referred to as primary prevention activities. Prevention is categorised into three levels
- Primary Prevention, which seeks to decrease the number of new cases of a disorder or illness. At this level of prevention we have:
- Health promotion/education, and
- Specific protective measures (such as immunisation)
- Secondary Prevention, which seeks to lower the rate of established cases of a disorder or illness in the population (prevalence). This level essentially involves measures that ensure early diagnosis (such as screening) and prompt management
- Tertiary Prevention, which seeks to decrease the amount of disability associated with an existing disorder. This level involves:
- Disability limitation and
- Rehabilitation
- The secondary and tertiary prevention activities focus on maintaining the health

of individuals with chronic conditions, delaying progression of their conditions, and preventing complications.

- Strategies for the Prevention of Communicable diseases
- Three core approaches - surveillance, outbreak investigations and immunization - are fundamental to the prevention of communicable diseases. While medicinal plants may appear to have limited role in these approaches, several medicinal plants and traditional medicines derived from them have been used to enhance immune response to several disease agents.

LITERATURE REVIEW

Plant name : *Hemedesmus indicus*

Hemedesmus indicus commonly known as anantmool or sariva belongs to family asclepiadaceae, well known drug in ayurvedic system of medicine. In Ayurveda the root of the plant is used as antipyretic, anti diarrhoeal, asthma, bronchitis, skin diseases, epilepsy, and abdominal distention[4]. Root decoction helps in various skin diseases, syphilis, loss of appetite and also for urinary disorders[5,6]. *Hemedesmus indicus* root also used by natives for treating ulcer. This plant is widely grown in several parts of the country.

Hemidesmus indicus, commonly known as Indian Sarsaparilla or Anantmool, is a plant species that is widely distributed in India, Pakistan, Sri Lanka, and other parts of South Asia. The plant belongs to the family Apocynaceae, which also includes other medicinal plants like Rauvolfia serpentina and Catharanthus roseus.

Hemidesmus indicus (L.) R.Br. ex Schult. belongs to the family Apocynaceae, which is further divided into several subfamilies. Hemidesmus indicus belongs to the subfamily Asclepiadoideae, which is characterized by the presence of milky sap, and pollen that is aggregated in waxy masses called pollinia.

Hemidesmus indicus is the only species in the genus *Hemidesmus*. However, there are several other closely related species that are sometimes classified as members of the same genus or placed in different genera within the subfamily Asclepiadoideae. Some of the closely related species are:

Hemidesmus maculatus (L.) R.Br. ex Schult.

Hemidesmus crinitus (L.) R.Br. ex Schult.

Hemidesmus fruticosus (L.) R.Br. ex Schult.

Hemidesmus hirsutus (L.) R.Br. ex Schult.

The plant has a long history of use in traditional medicine systems like Ayurveda and Unani. The roots of the plant are the most commonly used part and are known to possess a wide range of medicinal properties. The roots are rich in bioactive compounds like saponins, flavonoids, and alkaloids that are responsible for its therapeutic effects ,it is a family of flowering plants that includes around 5,000 species distributed in tropical and subtropical regions of the world. The family is characterized by the presence of milky sap, opposite or whorled leaves, and flowers with five petals and five sepals. Many species of the family Apocynaceae have medicinal properties and are used in traditional medicine for the treatment of various diseases.

Hemidesmus indicus has been used for the treatment of various ailments like fever, skin diseases, respiratory disorders, rheumatism, and digestive disorders. The plant is known to possess anti-inflammatory, analgesic, antioxidant, and immunomodulatory properties, which

make it useful in the management of these health conditions.

One of the most well-known uses of *Hemidesmus indicus* is as a blood purifier. The plant is believed to have the ability to detoxify the blood and remove toxins from the body. This property makes it useful in the treatment of skin diseases like eczema, psoriasis, and acne. The plant is also used for the treatment of other skin conditions like leprosy and scabies.

Hemidesmus indicus is also known for its anti-inflammatory properties. The plant has been found to inhibit the production of pro-inflammatory cytokines, which makes it useful in the treatment of inflammatory disorders like arthritis and asthma. The plant is also known to possess analgesic properties, which make it useful in the management of pain.

The plant is also used for the treatment of respiratory disorders like cough, bronchitis, and asthma. The roots of the plant are believed to possess expectorant properties, which help in the expulsion of mucus from the respiratory tract. The plant is also used for the treatment of digestive disorders like dyspepsia, flatulence, and constipation. The plant is believed to possess carminative and laxative properties, which help in the digestion of food and the elimination of waste from the body.

Hemidesmus indicus is also known for its immunomodulatory properties. The plant has been found to enhance the activity of the immune system, which makes it useful in the prevention and treatment of infections. The plant is also used for the treatment of cancer. The plant has been found to possess anticancer properties, which make it useful in the management of various types of cancer.

Hemidesmus indicus is a plant species with a wide range of medicinal properties. The plant has been used for the treatment of various ailments in traditional medicine systems for centuries. The roots of the plant are the most commonly used part and are known to possess anti-inflammatory, analgesic, antioxidant, and immunomodulatory properties. The plant is used for the treatment of various health conditions like skin diseases, respiratory disorders, digestive disorders, and cancer. However, before using the plant for medicinal purposes, it is important to consult a healthcare professional to avoid any adverse effects.

DPPH Method

(ii) Nitric oxide Scavenging Activity

(iii) Reducing Power Assay Method

DPPH (1, 1-diphenyl -2-picryl hydrazyl) Free Radical Scavenging Activity:

The molecule 1,1-diphenyl-2-picryl hydrazyl (α,α-diphenyl-βpicrylhydrazyl;DPPH) is characterized as a stable free radical by virtue of the delocalization of the spare electron over the molecule as a whole, so that the molecule does not dimerize, as would be the case with most other free radicals. The delocalization of electron also gives rise to the deep violet colour, characterized by an absorption band in ethanol solution centred at about 517 nm. When a solution of DPPH is mixed with that of a substrate (AH) that can donate a hydrogen atom, then this gives rise to the reduced form with the loss of violet colour. In order to evaluate the antioxidant potential through free radical scavenging by the test sample, the change in optical density of DPPH radicals is monitored.

The free radical scavenging activity of synthesized compound was measured by decrease in absorbance of ethanolic solution of DPPH. 0.1mM solution of DPPH in methanol was prepared. Gallic acid was taken as reference standard. Different concentrations of test sample (50mg/ml, 100mg/ml, 200mg/ml, and 400mg/ml) and standard (1.0µg/ml, 2.5 µg/ml, 5.0 µg/ml) were prepared using ethanol. 1.0 ml of 0.1mM DPPH solution was added to 3.0 ml of all concentrations of test and standard separately. These solutions were kept in dark for about 30min and the absorbances were measured at 517nm. Ethanol (3 ml) in the place of test sample was used as the blank. The capability to scavenge the DPPH radical was calculated using the following equation.

$$\text{DPPH Scavenged (\%)} = ((A_0-A_1)/A_0) \times 100$$

Where A_0 is the absorbance of the blank (containing all reagents except the test sample), and A_1 is the absorbance of test sample. The antioxidant activity of test sample was expressed as IC_{50} . The IC_{50} value is defined as concentration in (µg/ml) of test sample that scavenges free radicals by 50%.

Phosphomolybdenum Assay:

This assay is based on the reduction of Mo (VI) to Mo (V) by the sample analyte and subsequent formation of a green phosphate Mo (V) complex at acidic pH [4].

Different concentrations of test sample (50mg/ml, 100mg/ml, 200mg/ml, and 400mg/ml) and standard (1.0µg/ml, 2.5µg/ml, 5.0µg/ml) Gallic acid were prepared using suitable solvent. 0.3 ml of each concentration of test sample and standard was mixed with 3.0ml of reagent (0.6 M sulfuric acid, 28 mM sodium phosphate and 4mM ammonium molybdate). The tubes containing the reaction solution were capped and incubated at 95°C for 90 min. Then the absorbance of the solution was measured at 695 nm using UV-visible spectrophotometer after cooling to room temperature. Distilled water (0.3 ml) was used as blank in place of test sample.

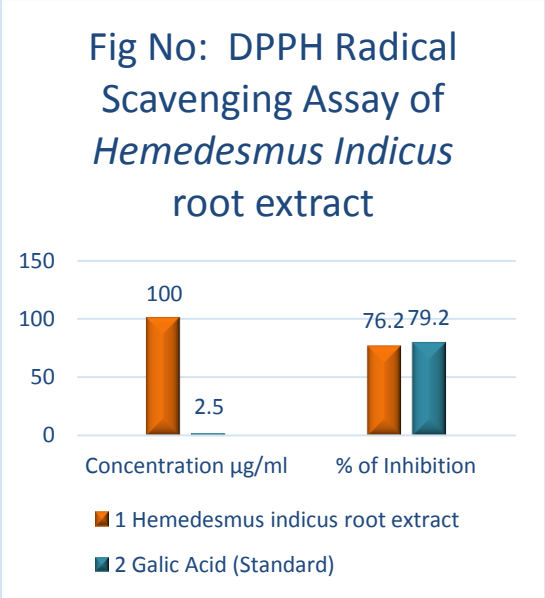
DPPH Radical Scavenging Assay[4]:

Hemedesmus Indicus root extract was subjected to DPPH radical scavenging assay, the extract at a concentration of 100ug/ml showed maximum inhibition is 76.20% and the results was compared with standard drug gallic acid 2.5 ug/ml 79.20%. The results are tabulated in table 2.

Table: DPPH Radical Scavenging Assay of Hemedesmus Indicus root extract

S.No.	Test	Concentration ug/ml	% of inhibition
1	*Hemedesmus Indicus* root extract	100	76.20 ± 0.48
2	Gallic Acid (Standard)	2.5	79.20 ± 0.60

Values are Mean + SEM n = 3



DPPH Radical Scavenging Assay of *Hemedesmus Indicus* root extract

Antioxidant Activity:

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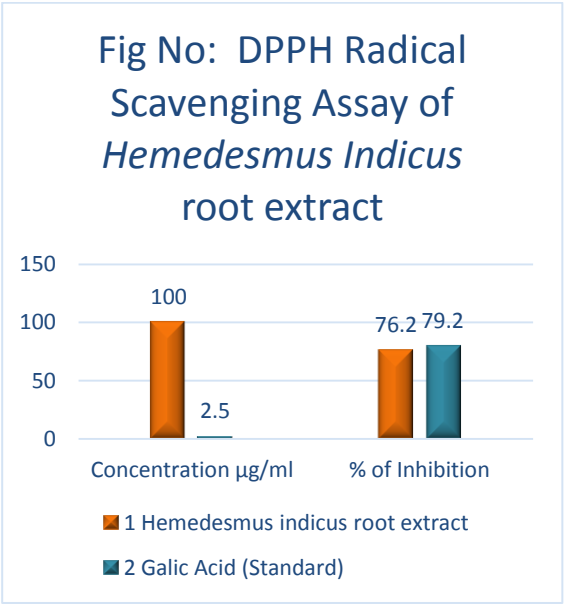

Figure - DPPH Radical Scavenging Assay of *Hemedesmus Indicus* root extract

Conclusion

In terms of antibacterial activity, the root extracts of both Hemedesmus indicus and Justicia Betonica demonstrated promising results in inhibiting the growth of bacteria. This suggests their potential application as natural antibacterial agents. The presence of bioactive compounds in these plants may contribute to their antimicrobial effects, which could be further explored to develop alternative treatments for bacterial infections.

The antioxidant activities of the root extracts were evaluated using various methods, including the DPPH method, nitric oxide scavenging activity, and reducing power assay. Both Hemedesmus indicus and Justicia Betonica extracts exhibited significant antioxidant potential, indicating their ability to scavenge free radicals and protect against oxidative stress-related damage. These findings

highlight the plants' potential as natural sources of antioxidants, which could have implications in preventing or managing various oxidative stress-related diseases.

The anti-inflammatory activities of the extracts were assessed using protein denaturation and HRBC membrane stabilization methods. Both Hemedesmus indicus and Justicia Betonica extracts demonstrated notable anti-inflammatory effects, suggesting their ability to inhibit inflammatory processes. These findings imply that these plant extracts may possess therapeutic potential in mitigating inflammatory conditions and could be explored further for the development of anti-inflammatory agents.

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