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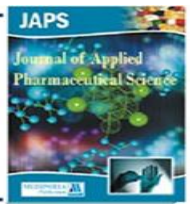

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Estimation of phytochemicals and antimicrobial activities of mentha spicata from southern districts of KPK

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ABSTRACT

The medicinal value of the plant is due to the phytochemical constituents they produce, which exhibit certain physiological actions on human body. Phytochemicals are plant derived chemical compoundS, which are non-essential nutrients, some of which show potential health promoting properties. The phytochemical constituents were determined by using known literature method while the antimicrobial activity was analyzed by classical literature methods. In case of phytochemicals, Tannin, Alkaloids, Glycosides, Flavonoids, Steroids, Coumarines, Sterols and Terpenes were found while saponins and anthraquinones were not determined in all the samples. Antibacterial activity was noted high in all the samples of crude extract followed by ethyl acetate and lowest activity was found was found in aqueous extract of *Mentha spicata*. The Same results were analyzed for antifungal activity.

Key words: Mentha spicata, southern districts, secondary metabolites, antbacterial, anti-fungal activity.

INTRODUCTION

The medicinal plants are reliable sources for the treatment of many health problems. Man has depended a lot on the herb in the past, and even at present the use of plants as medicine is popular. For the future health challenges the plants are reasonably prepared to serve the man. The only need is to develop the isolation and purification techniques. The medicinal value of the plant is due to the phytochemical constituents they produce, which exhibit certain physiological actions on human body. Phytochemicals are plant derived chemical compoundS, which are non-essential nutrients, some of which show potential health promoting properties. The phytochemicals are grouped into two main categories (Dharmesh, 2010), namely primary constituents which includes Amino acids, common sugar ,protein and chlorophyll etc and secondary constituents consists of Alkaloids, essential oils, Flavonoids, Tannins, Terpenoids, saponins, phenolic compounds etc (Akinmoladun et al, 2007) Majority of phytochemicals have been known to have valuable therapeutic activities like insecticidal ,antifungal, antibacterial, anticonstipative, spasmolytic, (Edeogo et al, 2005, Bisset, 1994) antispasmodial and antioxidant activities (Krishnaiah et al, 2007) etc. The plant thus finds medicinal value due to respective phytochemicals constituents they contain. (Dagne, 1996) Now days multiple drug resistance have been developed due to the indiscriminate use of commercial antimicrobial drugs used in the treatment of infectious diseases.

In addition to this problem, antibiotic are sometimes associated with adverse effects on the ,host including hypersensitivity, immune suppression, and allergic reactions. This situation forced scientist to reach for new antimicrobial substances.given the alarming incidence of antimicrobial resistance in bacteria of medical importance (Iwu et al, 1999, Benkeblia, 2004. There is a constant need for new and effective therapeutic agents; therefore there is a need to develop alternative antimicrobial drugs for the treatment of infectious diseases from medicinal plants (Gulcin et al, 2004; Senthilkumar, 2010).

MATERIALS AND METHODS

Extraction and Isolation

Simple extraction procedure was adopted for *Mentha spicata* Whole plant material dried under shade was chopped and pulverized into fine powder. 5.0 kg of dried powder was macerated with 80 % methanol three times at room temperature. Resulting methanolic extract (479.31 g) was evaporated under vacuum by rotary evaporator at 45 °C that afforded a gummy residue. The crude extract (470 g) was suspended in water and fractionated successively with n-hexane, chloroform, ethyl acetate and n-butano followed by leaving a residual water-soluble fraction. Each fraction was then concentrated using rotary evaporator at 50°C to yield n-hexane fraction (81.69g, 17.38%), chloroform fraction (108.97 g, 23.19%) and ethyl acetate fraction (79.14 g, 16.84%), the remaining was water fraction (196.82 g, 41.88%).(A)

Phytochemical Analysis

The crude methanolic extract of the plant material was tested for various classes of natural products using standard qualitative methods (Evans,2000, Tyler,1994, Harborne,1973). Following protocols were used for phytochemical tests, while results are summarized in table 1.

Tannins

The test for tannins was performed by subjecting 1 g plant extract in 2 ml distilled water, filtered and ferric chloride reagent was added to the filtrate. (Evans,2000, Tyler,1994, Harborne,1973)

Alkaloids

For alkaloids, the test was carried out by subjecting 1 g methanolic extract in 10 ml 1% HCl, boiled, filtered and Mayer's reagent was applied. (Evans,2000, Tyler,1994, Harborne,1973)

Saponins

The extract was subjected to frothing test for the identification of saponins. (Evans,2000, Tyler,1994, Harborne,1973)

Flavonoids

The presence of flavonoids was determined by using 1% aluminum chloride solution in methanol concentrated HCl, magnesium turnings, and potassium hydroxide solution. (Evans,2000, Tyler,1994, Harborne,1973).

Steroids

Steroids were screened by adding 1 ml of acetic anhydride to 0.25 g methanolic extract of each sample with 1 ml H_2SO_4. The color changed from violet to blue or green in some samples indicating the presence of steroids. (Evans,2000, Tyler,1994, Harborne,1973)

Anthraquinones

The test for anthraquinones was performed by adding 1 g of extract to 2ml benzene, filtered and ammonia solution added. (Evans,2000, Tyler,1994, Harborne,1973)

Coumarins

For detecting coumarins, a piece of filter paper was moistened in NaOH and then kept over a test tube with boiling plant extract solution. If the filter paper later showed any yellow fluorescence under UV light that was taken to indicate a positive test for coumarins. (Evans,2000, Tyler,1994, Harborne,1973)

Terpenes

Detection for any sterols and terpenes in the extract involved treatment of the extract with petroleum ether followed by extraction with $CHCl_3$. The subsequently acquired $CHCl_3$ layer was treated with acetic anhydride and concentrated HCl. The change of pink to purple and green to pink colors was indicative of the presence of terpenes or sterols, respectively. (Evans,2000, Tyler,1994, Harborne,1973)

Antimicrobial activity

The antibacterial activity was determined by using Agar Diffusion Method while the antifungal activity was analyzed using Agar Tube Dilution Assay(Hanna,2008, Lee,2003)

RESULT AND DISCUSSION

Phytochemicals

The medicinal value of the plants lies in bioactive phytochemical constituents that produce definite physiological actions on the human body. Some of the most important bioactive phytochemical constituents are Tannin, Alkaloids, Saponins, Flavounoids, Steroids, Anthraquinones, Coumarins and Sterols and Terpenes (Hanna,2008)

Table 1: Qualitative Analysis of Phytochemicals.

Phytochemical	Analysis			
	Peshawar	kohat	Karak	Bannu
Tannins	+	+	+	+
Alkaloids	+	+	+	+
Saponins	-	-	-	-
Flavonoids	+	+	+	+
Steroids	+	+	+	+
Anthraquinones	-	-	-	-
Coumarins	+	+	+	+
Sterols and Terpenes	+	+	+	+

Phytochemicals study were carried out qualitative for analysis of Tannin, Alkaloids, Flavounoids Steroids, Cumarinesand Sterols and Terpines which were identified in all districts (Table 1) including Peshawar,Kohat,Karak and Bannu saponins and

anthraquinones were not determined. .

Antibacterial Activity

sInfectious diseases are the leading cause of death worldwide, accounting for nearly one half of all deaths in tropical countries which are also becoming a significant problem in developed countries. In this regard plants can be provided a good alternative in search for new chemicals with a wide range of antibacterial and antifungal activities (Edeogo et al,2005)

Table 2: Antibacterial activity of extract and fraction *of Mentha spicata* collected from Peshawar in terms of Minimum Inhibitory Concentration (MIC, mg/mL).

Microorganism	Minimum Inhibitory Concentration (MIC, mg/mL)						
	Cipro	Cr	Hx	Cl	Et	Bu	Aq
Escherchia .coli	0.0002	1.75	-	2.0	1.75	1.75	-
Bacillus subtilis	0.0005	0.75	0.75	0.12	0.12	1.75	-
Shigella flexeneri	0.0003	0.25	1.75	0.06	1.75	1.75	1.75
Staphylococcus aureus	0.0009	0.75	-	0.5	1.75	0.75	1.75
Pseudomonas aeruginosa	0.0021	0.5	0.5	1.75	2.00	1.75	2.00
Salmonella typhi	0.0014	0.25	0.5	0.25	2.00	2.00	-

Cipro:ciprofloxacin, Cr: Crude extract, Hx: n-hexane, Cl:Chloroform, Et: Ethyle acetate, Bu:n-butanol, Aq: Aqueous

As can be seen from table 2, crude extract was found to be the most active extract. However in case of fractions, chloroform fraction was the second most active sample. It was followed by ethyl acetate, butanol and hexane fraction, while the Aqueous fraction showed the lowest activity among all samples.

Table 3: Antibacterial activity of the extract and fractions of *Mentha spicata* collected from Kohat in terms of Minimum Inhibitory Concentration (MIC, mg/mL).

Microorganism	Minimum Inhibitory Concentration (MIC, mg/mL)						
	Cipro	Cr	Hx	Cl	Et	Bu	Aq
Escherchia .coli	0.0002	0.25	-	0.12	0.75	0.75	-
Bacillus subtilis	0.0005	1.75	1.75	0.5	0.12	0.5	-
Shigella flexeneri	0.0003	0.25	2.0	1.75	1.75	2.25	1.75
Staphylococcus aureus	0.0009	1.75	-	0.25	0.5	0.50	2.00
Pseudomonas aeruginosa	0.0021	0.06	0.75	0.5	0.75	2.00	0.50
Salmonella typhi	0.0014	0.5	0.5	0.75	0.5	1.75	-

Cipro:ciprofloxacin, Cr: Crude extract, Hx: n-hexane, Cl:Chloroform, Et: Ethyle acetate, Bu:n-butanol, Aq: Aqueous

All of the test samples showed considerable antibacterial activity against the bacterial strains used in the study. As a whole, crude extract was found to be the most active extract. In case of fractions, chloroform fraction was the second most active sample followed by ethanol and butanol fraction. Lowest activity among all samples was found in aquous fraction.

Table 4 data shows the highest activity was detected in the crude extract However in case of fractions, ethanol fraction was the second most active sample followed by chloroform, butanol hexane and aqueous fraction.

Table 4: Antibacterial activity of the extract and fractions of *Mentha spicata* collected from Karak in terms of Minimum Inhibitory Concentration (MIC, mg/mL).

Microorganism	Minimum Inhibitory Concentration (MIC, mg/mL)						
	Cipro	Cr	Hx	Cl	Et	Bu	Aq
Escherchia .coli	0.0002	1.75	-	0.75	1.75	1.75	-
Bacillus subtilis	0.0005	0.75	2.00	1.75	0.50	1.75	-
Shigella flexeneri	0.0003	1.75	2.25	2.00	1.75	0.75	1.75
Staphylococcus aureus	0.0009	0.50	-	0.50	0.50	0.75	2.00
Pseudomonas aeruginosa	0.0021	0.50	0.75	0.75	0.75	2.00	2.00
Salmonella typhi	0.0014	1.75	2.00	1.75	1.75	1.75	-

Cipro:ciprofloxacin, Cr: Crude extract, Hx: n-hexane, Cl:Chloroform, Et: Ethyle acetate, Bu:n-butanol, Aq: Aqueous

Table 5: Antibacterial activity of the extract and fractions of *Mentha spicata* collected from Bannu in terms of Minimum Inhibitory Concentration (MIC, mg/mL).

Microorganism	Minimum Inhibitory Concentration (MIC, mg/mL)						
	Cipro	Cr	Hx	Cl	Et	Bu	Aq
Escherchia .coli	0.0002	0.25	-	0.25	0.75	1.75	-
Bacillus subtilis	0.0005	0.75	1.75	0.25	0.12	2.25	-
Shigella flexeneri	0.0003	0.50	2.25	2.00	1.75	0.75	0.50
Staphylococcus aureus	0.0009	0.12	-	0.50	0.50	2.00	2.00
Pseudomonas aeruginosa	0.0021	0.50	2.00	0.75	2.00	0.75	1.75
Salmonella typhi	0.0014	0.75	1.75	0.12	2.00	2.00	-

Cipro:ciprofloxacin, Cr: Crude extract, Hx: n-hexane, Cl:Chloroform, Et: Ethyle acetate, Bu:n-butanol, Aq: Aqueous

Crude extract was found to be the most active extract. However in case of fractions, ethanol fraction was the second most active sample. It was followed by chloroform, butanol and hexane fraction in a sequential order. Aqueous fraction revealed the lowest activity among all samples.

Table 6: Antifungal activity of the extract and fractions of *Mentha spicata* collected from Peshawar in terms of Minimum Inhibitory Concentration (MIC, mg/mL).

Microorganism	Minimum Inhibitory Concentration (MIC, mg/mL)						
	Clot	Cr	Hx	Cl	Et	Bu	Aq
Trichophyton longifusus	0.0014	1.75	-	3.5	2.25	0.75	3.5
Microsporum canis	0.0006	0.75	-	2.75	1.75	1.75	-
Candida albicans	0.0001	2.25	3.5	4.00	0.25	2.75	2.25
Aspervusgillus flavus	0.027	2.25	2.75	1.75	1.75	2.25	3.5
Fusarium solani	0.0011	0.75	-	1.75	3.5	-	3.5
Candida glaberata	0.0003	2.75	-	3.5	2.75	-	-

Clot:clotrimoxazole, Cr: Crude extract, Hx: n-hexane, Cl:Chloroform, Et: Ethyle acetate, Bu:n-butanol, Aq: Aqueous

The most active extract was found to be crude extract and in case of fractions, ethanol fraction was the second most active sample followed by butanol and chloroform fraction while Hexane fraction revealed the lowest activity among all samples All of the test samples showed considerable antifungal activity against the fungal strains used in the study. As a whole, Ethanol fraction was found to be the most active extract. However in case of fractions,

crude extract was the second most active sample. It was followed by chloroform ,butanol and Hexane fraction.

Table 7: Antifungal activity of the extract and fractions of *Mentha spicata* collected from Kohat in terms of Minimum Inhibitory Concentration (MIC, mg/mL).

Microorganism	Minimum Inhibitory Concentration (MIC, mg/mL)						
	Clot	Cr	Hx	Cl	Et	Bu	Aq
Trichophyton longifusus	0.0014	0.75	-	2.25	0.75	2.25	2.25
Microsporum canis	0.0006	2.75	-	2.75	2.75	2.75	-
Candida albicans	0.0001	1.75	2.75	4.00	0.50	2.75	4.00
Aspergillus flavus	0.027	2.25	3.50	2.25	0.75	3.50	2.75
Fusarium solani	0.0011	3.5	-	2.75	3.50	-	2.75
Candida glaberata	0.0003	0.50	-	2.25	0.75	-	-

Clot:clotrimoxazole, Cr: Crude extract, Hx: n-hexane, Cl:Chloroform, Et: Ethyle acetate, Bu:n-butanol, Aq: Aqueous

Table 8: Antifungal activity of the extract and fractions of *Mentha spicata* collected from Karak in terms of Minimum Inhibitory Concentration (MIC, mg/mL).

Microorganism	Minimum Inhibitory Concentration (MIC, mg/mL)						
	Clot	Cr	Hx	Cl	Et	Bu	Aq
Trichophyton longifusus	0.0014	0.75	-	2.25	1.75	1.75	2.75
Microsporum canis	0.0006	1.75	-	4.00	2.75	2.25	-
Candida albicans	0.0001	1.75	4.00	4.00	0.75	2.75	3.50
Aspergillus flavus	0.027	2.75	2.75	2.75	0.75	2.28	2.25
Fusarium solani	0.0011	3.50	-	2.25	3.50	-	3.50
Candida glaberata	0.0003	2.25	-	3.50	2.25	-	-

Clot:clotrimoxazole, Cr: Crude extract, Hx: n-hexane, Cl:Chloroform, Et: Ethyle acetate, Bu:n-butanol, Aq: Aqueous

Table 9: Antifungal activity of the extract and fractions of *Mentha spicata* collected from Bannu in terms of Minimum Inhibitory Concentration (MIC, mg/mL).

Microorganism	Minimum Inhibitory Concentration (MIC, mg/mL)						
	Clot	Cr	Hx	Cl	Et	Bu	Aq
Trichophyton longifusus	0.0014	0.50	-	2.25	0.50	1.75	2.75
Microsporum canis	0.0006	2.75	-	1.75	1.75	2.25	-
Candida albicans	0.0001	1.75	2.25	2.25	1.75	1.75	2.25
Aspergillus flavus	0.027	0.75	2.75	3.50	0.75	4.00	3.50
Fusarium solani	0.0011	2.25	-	2.75	2.75	-	1.75
Candida glaberata	0.0003	1.75	-	1.75	3.50	-	-

Clot:clotrimoxazole, Cr: Crude extract, Hx: n-hexane, Cl:Chloroform, Et: Ethyle acetate, Bu:n-butanol, Aq: Aqueous

Table 8 data shows that ethanol fraction was found to be the most active extract. However in case of fractions, crude extract was the second most active sample followed by chloroform butanol and Hexane fraction. Crude extract showed high activity seen from table 9. In case of fractions, ethanol fraction was the second most active sample while Hexane fraction revealed the lowest activity among all samples.

Approximately, 20% of the plants found in world have been submitted to pharmaceutical or biological test and sustainable number of new antibiotic introduced on the market are obtained from natural or semi synthetic resources . Chemical compound with antimicrobial activities isolated from plants have enormous therapeutic potential and are effective in the treatment of infectious diseases. Reports are available on the use of several plant by-products which possesses antimicrobial properties on several pathogenic bacteria and fungi (Byika et al,2004). So there is a continuous and urgent need to discover new antimicrobial compounds with diverse chemical structures and novel mechanism of action for new and re emerging infectious diseases .Therefore, researchers are increasingly turning their attention to herbal medicines looking for new leads to develop better drugs against microbial infections . All these facts shows that plant based antimicrobial agents possess vast untapped sources for drugs and have enormous therapeutic potential.(Benkeblia,2004)

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