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PHYTOCHEMICAL AND ANTITUBERCULAR SCREENING OF THE LEAF EXTRACTS OF *FOENICULUM VULGARE*

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ABSTRACT

Foeniculum vulgare is a perennial herb native to southern Europe and Mediterranean Sea. It has a wide range of biological potential which comprises of both pharmacological and antimicrobial perspectives. In the present investigation, the leaf extracts (pet ether, chloroform and methanol) of *Foeniculum vulgare* were been exposed to combat against *Mycobacterium tuberculosis* using BACT/ALERT TB assay method. Phytochemical Screening revealed the presence of secondary metabolites such as phenols, glycosides, terpenoids, alkaloids, saponins, flavanoids and tannins. Pharmacognostical investigation revealed the amount of inorganic salts present in the plant in the form of total ash content (10.53), acid insoluble ash (0.14) and total ash content (0.18) respectively. The corresponding total flavanoids content found in the chloroform extract, pet ether extract and methanol extract were found to be 0.186,0.049 & 0.086 respectively. *In vitro* antimycobacterial screening revealed that both pet ether and methanolic extracts Showed resistant against ***Mycobacterium tuberculosis***. On contrary, chloroform extract of *Foeniculum vulgare* showed antimycobacterial activity at the concentration of 60 mg/mL by BACT/ALERT TB assay method.

Key words: *Foeniculum vulgare, Mycobacterium tuberculosis*, BACT/ALERT TB method.

1. INTRODUCTION

India has the highest burden of tuberculosis in the world with over two million incident cases amounting to more than 5th of global burden. Tuberculosis has been known to have devasting effects on the socioeconomic development especially in the developing countries due to its association with the dreaded disease like HIV/AIDS. According to WHO 1990 report states that approximately 8 million cases out of which 7.6 new million cases were reported (Tuberculosis India, 2012).

Due to its prevailing multidrug resistant cases, it is a challenge for the medicinal chemists to combat and explore new drug molecules. In the present scenario, phytochemicals can also protect us against many microbial related diseases. They came as an alternative rescue force. In the present study, we have investigated a perennial herb (*Foeniculum vulgare*) commonly known as fennel which is a native of India, Republic china, Egypt and Pakistan. It has been well known by herbalists and alternative medical practitioners since time immemorial. It is one of the nine Anglo-Saxon sacred herbs. (Lillian Barros, et. al., 2010)

The folklore claims states that fennel seed has been possessing antispasmodic, carminative, diuretic, expectorant, laxative, stimulant and antiulcer properties (WHO, 2002). Fennel has also been used to stimulate lactation, as a remedy against colic disorders. Chinese herbal medicine explores the phytopharmacological aspects in the treatment of gastroenteritis, hernia, indigestion and abdominal pain. Further, ***Foeniculum vulgare*** (fennel) justifies antioxidant properties, nutritional perspectives (Filomena Confrorti et.al, 2006) and its biological potential in the treatment of respiratory diseases. With these prevailing properties, it is our interest to investigate and explore the leaf extracts of ***Foeniculum vulgare*** potential against ***Mycobacterium tuberculosis*** by BACT/ALERT assay method.

2. MATERIALS AND METHODS

2.1 PLANT MATERIAL

The Plant Specimen (***Foeniculum vulgare)*** for the proposed study was collected from Nacharam Village, Medak District, A.P. The plant was authenticated by Prof. P.Jayaraman, Plant Anatomy Research Centre, Chennai and authentication number is denoted as PARC/2013/2053. Care was taken to select the healthy plants and for normal organs such as leaves, bark, flowers, fruits and seeds. The required sample of leaves were isolated from the plant with exercising proper care such that other organs were not been damaged.

2.2 EXTRACTION OF THE PLANT MATERIAL

The leaves were washed with water and cut in to small pieces and shade-dried for a couple of weeks. The dried leaves were pulverized and passed through the sieve no-40 mesh. The powder thus obtained was employed for extraction (Harbone J, 1973).

2.3 PREPARATION OF PETROLEUM ETHER EXTRACT

The shade dried coarsely powdered leaves of ***Foeniculum vulgare*** (100 g) were extracted by cold maceration technique with petroleum ether for 72 hours. After the completion of the extraction, the solvent was removed by rota vap under reduced pressure. The percentage yield of pet-ether extract was found to be 0.7%w/w (Pale green extract).

2.4 PREPARATION OF CHLOROFORM EXTRACT

The shade dried coarsely powdered leaves of ***Foeniculum vulgare*** (100 g) were extracted by cold maceration technique with chloroform for 72 hours. The extract has been recovered by rota vap under reduced pressure. The percentage yield of chloroform extract was found to be 1.7 % w/w (Dark green extract).

2.5 PREPARATION OF METHANOLIC EXTRACT

The shade dried coarsely powdered leaves of ***Foeniculum vulgare*** (100 g) were further consecutively extracted with methanol by cold maceration technique for 72 hours. The extract has been recovered by rota vap under reduced pressure. The percentage yield of methanolic extract was found to be 0.8 % w/w (Dark green extract).

3. QUALITATIVE ANALYSIS OF DIFFERENT EXTRACTS OF *Foeniculum vulgare* LEAF POWDER

All the extracts (pet ether, chloroform and alcohol) of ***Foeniculum vulgare*** were subjected to the qualitative test to explore the various phytoconstituents present in their respective extracts.

3.1 Test for Steroids

1 mg of the extracts were dissolved in a few drops of chloroform, 3 ml of acetic anhydride and 3 ml of glacial acetic acid were added, warmed and cooled under the running tap water. Then add few drops of Conc. Sulphuric acid along the sides of the test tube. Appearance of green colour shows the presence of phytosterols.

3.2 Tests for Phenols

To the 1 mg of the extracts, add few drops of alcohol and ferric chloride. Bluish green or red colour indicates the presence of phenols.

3.3 Tests for Alkaloids

To the 1 mg of the extracts, add a few drops of acetic acid and Dragendroff's reagent and shaken well. Appearance of orange precipitate indicates the presence of alkaloids.

3.4 Tests for Flavanoids

To the 1 mg of extracts, add few magnesium turnings and few drops of Conc. Hydrochloric acid and boiled for few minutes. Presence of red colouration reveals the presence of flavones.

3.5 Tests for Tannins

To the 1 mg of extracts, add a basic lead acetate solution. Appearance of a white precipitate reveals the presence of tannins.

3.6 Tests for Glycosides

To the 1 mg of the extracts, mix equal quantity of Anthrone and Conc.Sulphuric acid. Heat the mixture gently on a water bath. Appearance of dark green colour indicates the presence of glycosides. The results were illustrated in table no: 1

4. 1DETERMINATION OF TOTAL ASH (QUALITY CONTROL METHODS, 2002)

Place about 2.4 g of the ground air dried material in a tarred crucible made up of silica and ignite it by gradually increasing the heat to 500°C-600°C until it is white indicating the absence of carbon. The material is cool in a dessicator and weigh. To the residue add 2 ml of water or a saturated solution of ammonium nitrate, dry on a water bath. Then on a hot plate, ignite to constant weight. Allow the residue to cool in a suitable dessicator for 30 minutes, and then weigh without delay.

4.2 DETERMINATION OF ACID INSOLUBLE ASH

To the crucible containing the total ash, add 25 ml of hydrochloric acid, cover with watch glass and boil gently for 5 minutes. Rinse the watch glass with 5 ml of hot water and add this liquid to the crucible. Collect the insoluble matter on ash less filter paper and wash with hot water until the filtrate is neutral. Transfer the filter paper containing the insoluble matter to

the original crucible, dry on hot plate and ignite to constant weight and allow the residue to cool in a suitable dessicator for 30 minutes and then weigh without delay.

4.3 DETERMINATION OF WATER SOLUBLE ASH

To the crucible containing total ash, add 25 ml of water and boil for 5 minutes. Collect the insoluble matter in a sintered glass crucible or ash less filter paper. Wash with hot water and ignite in a crucible for 15 minutes at a temperature not exceeding 450°C. Subtract the weight of the residue in mg from the weight of total ash. Calculate the content of water soluble ash in mg per gram of air dried material. The results of the ash values were been illustrated in the table no: 2

5. ESTIMATION OF TOTAL FLAVONOID CONTENT (RAJANANDH, 2010)

5.1 Preparation of Standard solutions

Quercetin is used as the standard for estimation of total flavonoids in the prepared extract.1mg of quercetin was dissolved in 100 ml of methanol to get 10 µg/ml solution.

5.2 Preparation of calibration curve

Aliquots of 0.1, 0.2, 0.4, 0.6, 0.8 and 1.0 ml from the above stock solution were taken in 6 different 10 ml volumetric flask. To each flask, add 1.5 ml of methanol, 0.1 ml of 10% aluminum chloride, 0.1 ml of 1M potassium acetate and 2.8 ml of distilled water was added. The reaction mixture was kept aside at room temperature for 30 minutes and the volume is making up with the water. The absorbance of the resulting solution was measured at 415 nm against reagent blank. The calibration curve was prepared by plotting absorbance vs. concentration and it was found to be linear over this concentration range of 1-10 µg/ml.

5.3 Preparation of test solutions

1mg of the extract was dissolved in 100 ml of methanol to get 10 µg/ml solution. Required volume of the above solution was transferred in to a 10 ml standard flask and color development was carried out as that of standard. Absorbance of the test solution was measured at 415 nm against blank. The concentration of total flavonoids in the test sample was determined by extrapolation from the calibration curve. The total flavonoid content in the extracts was expressed as µg/mL. The results were tabulated on table no: 3

6. Antimycobacterial screening using BacT Alert 3D method (*Mycobacterium* support, 2009)

The Bact/Alert 3D system deliver the high performance detection of positive results as they occur. Based on biomeriux proprietary colorimetric sensor technology, each culture bottle is continuously monitored for organism growth by the highly sensitive reflectometer. The Bact/Alert 3D capabilities in a compact format a control and incubation module in a single instrument. The system used advanced colorimetric detection of carbon dioxide reduction and a sophisticated computer algorithm for a fast time to detection of positive growth (98% of all positive growth was detected within 72 hours). The Light emission sensor (LES) at the bottom of each bottle undergoes a color change from blue to yellow. This change is permanent and visible indicating the growth of the organism.

For Mycobacterial testing, the mycobacterial culture bottles contain 10 ml of Middle Brook 7H9 media is employed. An antibiotic supplement is added to each bottle to prevent bacterial or fungal growth. The supplement contains Polmyxin B, Azlocillin, Nalidixic acid, Trimethoprim, Amphotericin, Vancomycin and growth factors. The middle brook bottles (which contain RBC corpuscles- Blood) for the culture and growth of mycobacteria contains an anticoagulant as well as lytic agent. To the Middle brook vials, add 1mL of the leaf extracts of *Foeniculum vulgare* has been incorporated at the corresponding concentration of 40 mg/ml, 60 mg/ml & 80 mg/ml respectively. To this mixture, Add 0.5mL of suspension of ***Mycobacterium tuberculosis*** culture (clinical isolate) and incubate at 37°C+/-1°C for 24 hours. The presence of growth of ***Mycobacterium tuberculosis*** and the drug sensitivity is been monitored by BacT Alert 3D screening instrument. When the drug is sensitive, the emission of light emitting sensor (LES) remains blue color implementing there is no growth of ***Mycobacterium tuberculosis***. The appearance of yellow emission reveals there is a growth of ***Mycobacterium tuberculosis*** complex in the culture bottle, which in turn indicates that the drug is resistant. The results were tabulated in the table no: 4.

7. RESULTS AND DISCUSSION

The Preliminary Phytochemical studies explore the presence of alkaloids, glycosides, tannins, flavanoids, phenols and terpenoids. The study made Patrica et al (2012) reveals the presence of fatty acids, glycosidal saponins and coumarins. The results of the phytochemical exploration the leaf extracts of ***Foeniculum vulgare*** were given in table no:1.

Table 1.

S.No	Chemical Test	Petroleum Ether Extract	Chloroform Extract	Methanol Extract
1	Alkaloids	-	+	+
2	Glycosides	+	+	+
3	Tannins	+	+	+
4	Flavanoids	+	+	+
5	Phenols	+	+	-
6	Coumarins	-	-	-
7	Triterpenes	+	-	+

Keys: - + = Present; - = Absent.

Results obtained from the ash value estimation has been tabulated in table no: 2. The leaf powder of ***Foeniculum vulgare*** has more inorganic salts out of which some were water soluble and acid insoluble in nature.

Table 2.

S.No	Total ash value	Water soluble ash	Acid insoluble ash
1	10.53	0.18	0.14

Results obtained from the total flavanoids content in table no: 3 reveal that the chloroform extract of ***Foeniculum vulgare*** has more flavanoid content compare to petroleum ether and methanol extracts.

Table 3.

S.No	Concentration µg/ml	Absorbance
1	1	0.021
2	2	0.043
3	4	0.091
4	6	0.136
5	8	0.168
6	10	0.198
7	Chloroform extract	0.186
8	Petroleum ether extract	0.049
9	Methanol extract	0.086

The study of antimycobacterial perspectives of the leaf extracts of ***Foeniculum vulgare*** dealt with the clinical strain of ***Mycobacterial tuberculosis*** reveals that chloroform extract of ***Foeniculum vulgare*** possess antimycobacterial effect against the clinical strain of ***Mycobacterial tuberculosis*** is studied by BacT method. The minimum inhibitory concentration (MIC) of Chloroform extract is found to be 60mg/mL. On contrary, petroleum ether and methanol extracts of ***Foeniculum vulgare*** showed resistance against ***Mycobacterial tuberculosis***. On the studies of Patrica et.al (2012) revealed that the antimycobacterial perspectives of ***Foeniculum vulgare*** (Mexico variety). The Hexane extract fractions offer excellent bioactive molecules against multidrug resistant ***Mycobacterial tuberculosis.*** In similar lines, Patrica et.al (2012) reported 5-Hydroxyl coumarin derived from chloroform extract reported to produce good antimycobacterial activity. The results were tabulated in the table no.4. Henceforth our study complies with the study of Patrica et.al (2012) who had investigated the antimycobacterial perspectives.

Table no: 4 In-vitro screening of the leaf extracts of *Foeniculum vulgare* against *Mycobacterial tuberculosis* by BacT Alert 3D TB Method.

S.No	Name of the extract	40 mg/ml	60 mg/ml	80 mg/ml
1	Petroleum ether extract	++++	++++	++++
2	Chloroform extract	++++	_____	_____
3	Methanol extract	++++	++++	++++

++++ = Positive Growth- Resistant _____ = Negative growth-Sensitive

7. CONCLUSION

The present study supports the fact that chloroform extract of ***Foeniculum vulgare*** offers an optimistic opportunity to explore the active molecules against ***Mycobacterium tuberculosis***. The dietary intake of fennel may lower the risk of ***Mycobacterium tuberculosis*** infection. This study reveals the first report of the antimycobacterial perspectives of chloroform extract of leaves of ***Foeniculum vulgare.***

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