

**IN VITRO ANTIFUNGAL, ANTIBACTERIAL AND ANTIOXIDANT
ACTIVITY OF ESSENTIAL OIL FROM THE AERIAL PARTS OF
AGRIMONIA AITCHISONII SCHONBECK TEMESY FROM
HIMALAYAN REGION**

**Mohammad S. Javed^{1*}, Pawan Kumar¹, Rishendra Kumar², A. K. Tiwari³ and
K. S. Bisht³**

¹Department of Chemistry, D.S.B. Campus Kumaun University, Nainital 263002,
Uttarakhand, India.

²Department of Biotechnology, Bhimtal Campus, Kumaun University, Nainital 263002,
Uttarakhand, India.

³Department of Plant Pathology, College of Agriculture, G. B. Pant University of Agriculture
& Technology, Pantnagar 263145, Uttarakhand, India.

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***Correspondence for
Author**

Mohammad S. Javed

Department of Chemistry,
D.S.B. Campus Kumaun
University, Nainital
263002, Uttarakhand,
India.

ABSTRACT

The essential oil from the aerial parts of *Agrimonia aitchisonii* Schonbeck Temesy growing in the Himalayan region is evaluated for its antifungal, antibacterial and antioxidant activity. The oil has shown 100% mycelia growth inhibition against *S. sclerotiorum*, *A. tenuis*, *C. graminicola* and *F. oxysporum* at a concentration of 2000µg/ml, 2000µg/ml, 3000µg/ml and 3000µg/ml respectively. However *R. solani* was found less susceptible to the oil of *Agrimonia aitchisonii*. The IC₅₀ values showed a range from 41.10µg/ml – 85.44µg/ml as compared to standard fungicides with IC₅₀ values 32.8µg/ml – 98.6µg/ml. The spore germination inhibition test has shown the IC₅₀ values as 101.4µg/ml, 314.74µg/ml and 684.7µg/ml for *A. tenuis*, *C. graminicola* and *F. oxysporum* respectively. The ZOI for the test

bacterial strains at a concentration of 500µg/ml were Gram negative bacteria *Pseudomonas aeruginosa* 424 (7mm), *Escherichia coli* 443 (4mm), *Aeromonas hydrophila* 646 (8mm) and Gram positive bacteria *Bacillus subtilis* 441 (4mm) and *Staphylococcus aureus* 737 (6mm). The oil has shown 435.7 ± 0.004 mg/ml gallic acid equivalent per 100mg of the oil as total phenolic content and 423 ± 0.003 mg/ml quercetin equivalent per 100 mg of the oil as total

flavonoid content. The VEAC values were found to be $0.454 \pm 0.003\text{g/L}$ and $0.538 \pm 0.001\text{g/L}$ for the essential oil and ascorbic acid respectively.

KEYWORDS: *Agrimonia*, essential oil, ailments, antioxidant, antibacterial activity, infections.

1. INTRODUCTION

Medicinal plants are renewable natural resources, providing valuable natural products which are often used in the treatment of various ailments and diseases caused by micro-organisms^[1] and are bestowed with several biologically active compounds which show different activity like antimicrobial, analgesic, anticancer, antipyrexial and antihypertensive activity.^[2,3,4] Herbs and spices are generally considered safe and proved to be effective against certain ailments and complications.^[5] *Agrimonia aitchisonii* belongs to family Rosaceae, the name agrimony was derived from (agremone) a word given by Greeks to the plants which has healing capability for eyes, found widely distributed at an altitude of 2100-2400m in Uttarakhand region in India.^[6] *Agrimonia aitchisonii* is found rich in oxygenated monoterpenes with methyl myrtenate (62.4%) as major compound.^[7] Marked antibacterial activity against *Staphylococcus aureus* and α -haemolytic streptococci has been reported for *Agrimonia*.^[8] Another specie *Agrimonia pilosa* have been reported to have biologically active and radical scavenging extracts.^[9,10] The methanolic extracts from root and leaves of *Agrimonia pilosa* showed potent antibacterial activity.^[11] *Agrimonia eupatoria* exerts antibacterial, antiviral, antitumour, analgesic, antioxidant, immunomodulatory, antidiabetic, gastrointestinal, hepatoprotective, wound healing and many other Pharmacological effects.^[12] There is no report on the bioactivity and antioxidant activity of essential oil of *Agrimonia aitchisonii* from kumaun Himalayan region, Uttarakhand, India. So, the objective of this study was aimed at determining the bioactive and antioxidant potential of the essential oil of *Agrimonia aitchisonii* from this region.

2. MATERIALS AND METHODS

2.1. Plant material

The Plant material (Whole aerial parts of *Agrimonia aitchisonii* Schonbeck Temesy) was collected from Lariakanta area of Nainital, Uttarakhand (2100m-2400m) during month of October 2014 when the plant was in its flowering stage. The Plant specimen was primarily identified by Dr. Y.P.S. Pangtey, Botany Department, Kumaun University, Nainital. The botanical identity was further confirmed by Botanical Survey of India (Dehradun). The

herbarium specimen [Herbarium No. BSI/NC9(7)/2003-04/Tech./717] has been deposited in the Herbarium of Botanical Survey of India, Northern Regional Centre, Dehradun (BSD).

2.2. Extraction of the Essential oil

The essential oil was obtained by steam distillation of fresh plant material (5kg Aerial parts) using a copper still fitted with spiral glass condenser. The distillate was saturated with NaCl and extracted with hexane. The hexane extract was dried using anhydrous sodium sulphate (Na_2SO_4) and the solvent was removed with a rotovap at aspirator pressure and 36°C temperature to yield 3g of the oil (0.06% by weight).

2.3. Plant pathogenic fungi

The foliage born and soil born fungi (table1) were obtained from the Department of Plant Pathology, College of Agriculture, G. B. Pant University of Agriculture & Technology, Pantnagar, India. The pure culture of these pathogenic fungal species were maintained on Potato Dextrose Agar (PDA) and stored at temperature below 4°C for further activity.

2.3.1. Preparation of pure culture and spore suspension

The Pathogenic fungi namely *Sclerotinia sclerotiorum*, *Alternaria tenuis*, *Colletotrichum graminicola*, *Rhizoctonia solani* and *Fusarium oxysporum f.sp. ciceris* were cultured on Potato Dextrose Agar (PDA) medium in sterilized Petri dishes (80mm in diameter). The 8 days old cultures of respective pathogens were used for harvesting spores in 10ml autoclaved distilled water using an inoculation loop. To obtain homogenous spore suspension, the suspension was centrifuged (Megafuge 1.0, Heracus Sepatech, Germany) at 1800rpm for 5 minutes. This suspension was serially diluted up to 10^{-2} dilution to obtain countable spores (about 250-500 spores) on center large square of hemocytometer (B.S 748, I.S 10269, Rohem, India). The spore of each fungus were counted with hemocytometer and concentration (spores/ml) (table 1) in each plate was calculated using formula

$$\text{Spores per ml} = n \times 25 \times 10^4$$

Where n is the average no. of spores in medium square (0.04 mm^2) of centered big square.

Serial concentrations of the oil were prepared by dissolving required amount of oil in 10% DMSO.

Table 1: Pathogenic fungi used for antifungal activity.

Pathogen	Host	conc. Spores/ml
<i>Sclerotinia sclerotiorum</i>	Mustard	NA
<i>Alternaria tenuis</i>	Brinjal	3.4×10^6
<i>Colletotrichum graminicola</i>	Sorghum	6.3×10^6
<i>Rhizoctonia solani</i>	Rice	NA
<i>Fusarium oxysporum</i>	Chick pea	4.2×10^6

NA=Not appeared.

2.3.2. In Vitro antifungal assay

Poisoned food technique^[13], using Potato Dextrose Agar (PDA) as medium, was used to check the antifungal activity of the oil against test fungi. The different concentrations of the essential oil were prepared by dissolving the appropriate amount of the oil in 10% DMSO and distilled water and then added into 20ml PDA to obtain desired concentration.^[14] Mycelia plugs (2mm in diameter) from the edges of each culture were placed in the centre of each PDA plate (80mm). The control sets were prepared using equal amounts of 10% DMSO only and no oil. The prepared plates were inoculated aseptically with assay discs of the test fungus and were incubated at $25 \pm 2^{\circ}\text{C}$ for 3-8 days until the growth in the control plates reached the periphery of the each plate. Growth inhibition of each fungal strain was calculated as the percentage inhibition of radial growth relative to control, using formula

$$\% \text{ mycelia inhibition} = \frac{C-T}{T} \times 100$$

Where C- is the radial growth in control plate and T- is the radial growth in the test plate.

The plates were used in triplicate for each treatment.^[15] IC₅₀ (concentration that produces a 50% inhibitory effect) values were graphically obtained from the dose response curves based on measurements at six different concentrations.

2.3.3. Determination of minimum inhibitory concentration (MIC)

The MIC of the oil was determined by agar dilution method.^[16] The oil sample was dissolved in 10% DMSO, a 10 μl spore suspension (approx 10^6 spores/ml) of each fungal strain was inoculated in the test tube in PDB medium and incubated for 4-8 days at $25 \pm 2^{\circ}\text{C}$. The control tubes containing PDB medium were inoculated only with fungal suspension. Where MIC is the minimum concentration of the oil in $\mu\text{g/ml}$ at which no visible growth was observed.

2.3.4. Spore germination assay

Spore germination assay^[17], for *A. tenuis*, *C. graminicola* and *F. oxysporum f.sp. ciceris* with some modifications was done at different concentrations of oil. Aliquots of 30µl of essential oil solutions at different concentration (250-2000 µg/ml) were mixed with 30µl 5% dextrose solution and 40µl of the spore suspension (approx 10⁶ spores/ml) in cavity slide which were incubated in a moist chamber at 25±2⁰C for 24 hours. Each slide was then fixed in Lactophenol-cotton blue and observed under microscope for spore germination. The spores that produced germ tubes were enumerated and percentage of spore germination was calculated in comparison with control assay. Each assay was performed in triplicate and the results were obtained as average of the three results. The control having only 10% DMSO was tested separately for spore germination of different fungi. The percent spore germination was calculated by using formula

$$\% \text{ spore germination} = \frac{Ng}{Nt} \times 100$$

Where Ng is the number of spores germinated and Nt is the total number of spores examined.

2.4. Determination of Antibacterial activity

2.4.1. Source of tested organisms

Pure cultures of all microorganisms were obtained from IMTECH (Institute of Microbial Technology), Chandigarh, India. Microorganisms (bacteria) selected for study were

Gram negative – *Pseudomonas aeruginosa* (M.T.C.C.No.424), *Escherichia Coli* (M.T.C.C. No. 443), *Aeromonas hydrophila* (M.T.C.C. No. 646).

Gram Positive – *Bacillus subtilis* (M.T.C.C. No.441) and *Staphylococcus aureus* (M.T.C.C. No.737).

2.4.2. Preparation of Inoculums

Nutrient agar (NA) and Nutrient Broth (NB) were used for culturing the bacteria. Inoculums were prepared by picking respective bacterial colony with the help of loops and pour into NB then incubated at 28⁰C for 48 hours for bacteria growth in shaking condition.

2.4.3. Antibacterial activity

The antibacterial activity was determined by using Agar well diffusion method.^[18] It was expressed as the mean of ZOI diameters (mm) produced by the essential oil. For screening

plates were prepared by using nutrient agar. The inoculums (90 μ l) of different bacterial strains were spread evenly on respective plates with sterile spreader and a borer (8mm diameter) was used to cut well. 20 μ l of different concentrations 5mg/ml, 10mg/ml, 15mg/ml, 20mg/ml (100 μ g/well, 200 μ g/well, 300 μ g/well, 400 μ g/well) and 25mg/ml (500 μ g/well) respectively, of the oil were poured in each well and incubated at 28^oC for 48hours. Control (Negative control) well contained 20 μ l of 5% DMSO in place of oil solution. The diameter of ZOI was measured and the mean was recorded. Experiment was performed in triplicate.

2.5. Determination of total Phenolic content (TPC)

The total phenolic content in the essential oil was determined by Folin-Ciocalteu's method^[19] with certain modifications. 1ml of the test sample was mixed with 1ml Folin-Ciocalteu's reagent and 0.8ml Na₂CO₃ (7.5%). The Mixture was kept at room temperature for 30 min and then absorbance was measured at 765 nm. Quantification of total phenolic content was based on standard curve of Gallic acid prepared in the two solvents respectively. The results were expressed in mg Gallic acid equivalent (GAE) per gram dry weight of the material.

2.6. Determination of Total Flavonoid Content

The total flavonoid contents were determined spectrophotometrically.^[20] In brief, 1ml of the sample was mixed with 75 μ l, 5% of NaNO₂ solution. The mixture was allowed to stand at room temperature for 5 minutes. Then 75 μ L of AlCl₃.6H₂O (10%) solution was added to it and allowed to stand for 5 minutes. Then 0.5 ml of 1M NaOH solution was added to the above solution and shaken vigorously and allowed to stand for 15 minutes at room temperature. After 15 minutes the absorbance was obtained in spectrophotometer at a Wavelength of 510nm.

Quantification of total flavonoid content was done on the basis of standard curve of quercetin prepared in the two solvents and the results were expressed in mg quercetin equivalent (QE) per gram dry weight of the material.

2.7. Determination of antioxidant activity

2.7.1. Determination of Ferric reducing antioxidant power (FRAP) Assay

It is one of the simple, direct tests for measuring of antioxidant capacity. It provides a measure of the reducing ability of the essential oil. The total antioxidant and potential of the oil was determined by using ferric reducing ability (FRAP) assay,^[21] as measure of antioxidant power. The FRAP reagent was freshly prepared by mixing 10mM 2,4,6-

tripyrindyltriazine (TPTZ) and 20mM ferric chloride in 0.25M acetate buffer, PH 3.6. 100 μ l of the test sample was added to 300 μ l of distilled water followed by 3ml of FRAP reagent. The absorbance was measured at 750nm after 5 min incubation at room temperature against a blank. The standard curve of tocopherol was constructed. Antioxidant power could be determined from the standard curve as Vitamin E Equivalent antioxidant capacity. Antioxidant activity of the sample was compared with standard ascorbic acid. This assay measures the change in absorbance at 750nm owing to the formation of a blue colored Fe²⁺-tripyrindyltriazine compound from colorless oxidized Fe³⁺ form by the action of electron donating antioxidants. The present data are average of three replications.

2.8. Statistical Analysis

For all tests, the mean values and standard deviations were calculated. The data were analyzed using SPSS 16.0 statistical software. The one-way analysis of variance (ANOVA) was applied for calculating results. The means were compared by Duncan tests at a level of significance of $P < 0.05$.

3. RESULTS

3.1. In vitro antifungal activity

After an incubation period of 4 days for *S. sclerotiorum*, *R. solani* and 8 days for *A. tenuis*, *C. graminicola*, and *F. oxysporum f. sp. ciceris* at $25 \pm 2^{\circ}\text{C}$ the effect of different concentrations of the oil from *Agrimonia aitchisonii* are summarized in table 3. The results show significant activity in comparison to fungicides (positive control). The oil inhibits the growth of mycelia strains in a dose dependent manner. The essential oil showed a varying effect at different concentrations. The oil was found effective against all the pathogenic test fungi. The inhibitory effect of the oil varied from 11.11% to 100% (table 2). The oil of *A. aitchisonii* completely inhibited the mycelial growth of *S. sclerotiorum*, *A. tenuis*, *C. graminicola* and *F. oxysporum f. sp. ciceris* at a concentration of 2000 μ g/ml, 2000 μ g/ml 3000 μ g/ml and 3000 μ g/ml respectively. However complete mycelia growth inhibition was not observed for *R. solani*. The IC₅₀ and MIC values of the oil from *A. aitchisonii* in comparison to fungicide (positive control) showed a marked effect (table 3).

3.2. Spore germination assay

The results shown by the essential oil against percent spore germination are summarized in table 5. The oil effected the germination of spores differently at different concentrations. The spore germination was not inhibited by Dimethyl sulphoxide (DMSO, 10% v/v) used as

control. The spore germination inhibition ranges from 38.65% to 100% for the test pathogens. The spore germination was completely inhibited for *A. tenuis* and *C. graminicola* at a concentration of 2000µg/ml and 1000µg/ml. The *F. oxysporum* spores were found slightly less susceptible to the essential oil of *A. aitchisonii* at the test range concentration. The IC₅₀ values for the spore germination of these pathogenic fungi were found 274.4µg/ml, 215.7µg/ml and 684.7µg/ml for *A. tenuis*, *C. graminicola* and *F. oxysporum* respectively where as MIC values appeared for *A. tenuis* and *C. graminicola* and are found to be 2000µg/ml and 1000µg/ml (Table 5). The percentage spore germination inhibition by the oil on comparison with the fungicides (positive control) showed a significant effect (table 5).

Table 2: % mycelia growth inhibition^a by essential oil from *A. aitchisonii*.

Conc. µg/ml → Pathogenic fungi ↓	100 µg/ml	250 µg/ml	500 µg/ml	1000 µg/ml	2000 µg/ml	3000 µg/ml
<i>S. sclerotiorum</i>	28.75	76.25	83.75	93.75	100.00	100.00
<i>A. tenuis</i>	41.66	66.66	76.66	83.33	100.00	100.00
<i>C. graminicola</i>	11.11	48.14	59.25	68.51	81.00	100.00
<i>R. solani</i>	55.00	56.25	66.25	71.25	80.21	93.15
<i>F. oxysporum</i>	32.50	43.75	60.00	70.00	87.50	100.00

^a values within columns are given as mean ± S.D. of three experiments.

Table 3: IC₅₀ and MIC values of essential oil and fungicides (positive control) against test pathogens.

Pathogenic Fungi	Essential oil		Fungicide (positive control)			
	IC ₅₀ ^a	MIC ^b	Carbendazim		Mancozeb	
			IC ₅₀ ^a	MIC ^b	MIC ^b	IC ₅₀ ^a
<i>S. sclerotiorum</i>	55.91	2000	32.8	500	NA	NA
<i>A. tenuis</i>	54.43	2000	39.3	NA	NA	NA
<i>C. graminicola</i>	85.44	3000	NA	NA	500	500
<i>R. solani</i>	55.97	NA	49.6	600	NA	NA
<i>F. oxysporum</i>	41.10	3000	NA	68.4	NA	NA

NA = Not applicable

^aConcentration (µg/ml) that produces a 50% inhibitory effect on radial mycelia growth.

^bMinimum inhibitory concentration (µg/ml).

Table 4: % spore germination inhibition by essential oil from *A. aitchisonii*.

Conc. $\mu\text{g/ml}$ \rightarrow Pathogenic fungi \downarrow	250 $\mu\text{g/ml}$	500 $\mu\text{g/ml}$	1000 $\mu\text{g/ml}$	2000 $\mu\text{g/ml}$
<i>A. tenuis</i>	40.35	68.66	85.55	100.00
<i>C. graminicola</i>	51.25	81.65	100.00	100.00
<i>F. oxysporum</i>	38.65	41.25	59.75	81.25

Table 5: IC₅₀ and MIC values of essential oil and fungicides (positive control) against spore germination of test pathogens.

Pathogenic Fungi	Essential oil		Fungicide (positive control)			
	IC ₅₀ ^a	MIC ^b	Carbendazim		Mancozeb	
			IC ₅₀ ^a	MIC ^b	MIC ^b	IC ₅₀ ^a
<i>A. tenuis</i>	101.4	1000	68.3	750	NA	500
<i>C. graminicola</i>	314.7	NA	NA	NA	38.2	500
<i>F. oxysporum</i>	684.7	NA	NA	NA	NA	NA

NA = Not applicable

^aConcentration ($\mu\text{g/ml}$) that produces a 50% inhibitory effect on spore germination.

^bMinimum inhibitory concentration ($\mu\text{g/ml}$).

3.3. Antibacterial activity

The results of the antibacterial efficacy of the oil from the aerial parts of *A. aitchisonii* are presented in table 6. The potency of the oil was tested for both Gram negative and Gram positive bacteria. The maximum ZOI was shown against *A. hydrophila* (8mm) and *P. aeruginosa* (7mm) at a concentration of 500 $\mu\text{g/ml}$. The oil has shown significant effect against both these strains and lowest effect against *E. coli* and *B. subtilis*. The oil has shown its effect in a dose dependant manner while the control which was 20 μl of 10% DMSO only, didn't have good antibacterial activity on these tested bacterial strains.

Table 6: ZOI shown by essential oil from the aerial parts of *A. aitchisonii* against different bacterial strains.

Conc. \rightarrow Bacterial strain \downarrow	100 $\mu\text{l/ml}$	200 $\mu\text{l/ml}$	300 $\mu\text{l/ml}$	400 $\mu\text{l/ml}$	500 $\mu\text{l/ml}$
<i>P. aeruginosa</i>	00mm	01mm	03mm	04mm	07mm
<i>E. coli</i>	01mm	01mm	02mm	02mm	04mm
<i>A. hydrophila</i>	01mm	03mm	03mm	05mm	08mm
<i>B. subtilis</i>	00mm	01mm	03mm	04mm	04mm
<i>S. aureus</i>	01mm	03mm	03mm	05mm	06mm

3.4. Total phenolic contents

Phenolic compounds may directly contribute to the antioxidant activity of the essential oil. The total phenolic content in this oil was 435.7 ± 0.004 mg/ml gallic acid equivalent per 100mg of the oil.

3.5. Total flavonoid content

Flavonoids present in the essential oil of the *A. aitchisonii* may also contribute towards antioxidant activity. The total flavonoid content of the oil was 423 ± 0.003 mg/ml quercetin equivalent per 100 mg of the oil.

3.6. Ferric reducing antioxidant power (FRAP) assay

The reducing ability of the essential oil was evaluated on the basis of VEAC (Vitamin E Equivalent Antioxidant Capacity) where the standard curve of the FRAP assay of tocopherol (Vitamin E) was plotted as absorbance versus different concentrations after 5 minutes (Fig.1). The antioxidant potential of the essential oil was assessed in the same way (Fig.2) and compared with that of ascorbic acid (Fig.3). VEAC values were calculated using appropriate equations and were found to be 0.454 ± 0.003 g/l and 0.538 ± 0.001 g/l for the essential oil and ascorbic acid respectively. The FRAP values for the essential oil are quite compare able to that of the ascorbic acid. The antioxidant power shows good linear relation in both standard ($R^2 = 0.948$) as well as sample oil ($R^2 = 0.963$) (Fig. 3, 2).

Table 7: Absorbance shown by the sample and control (Ascorbic acid) at different concentrations.

Concentration	Absorbance		
	Sample	control (ascorbic acid)	Vitamin E
100µl/ml	1.145 ±0.021	0.350 ±0.001	0.875±0.00
200µl/ml	2.138±0.003	1.830±0.012	2.135±0.00
300µl/ml	3.421±0.011	3.960±0.006	4.013±0.00
400µl/ml	3.620±0.002	4.330±0.014	5.673±0.00
500µl/ml	4.574±0.003	5.354±0.002	7.986±0.00

Values in the table are mean of the three results \pm SD.

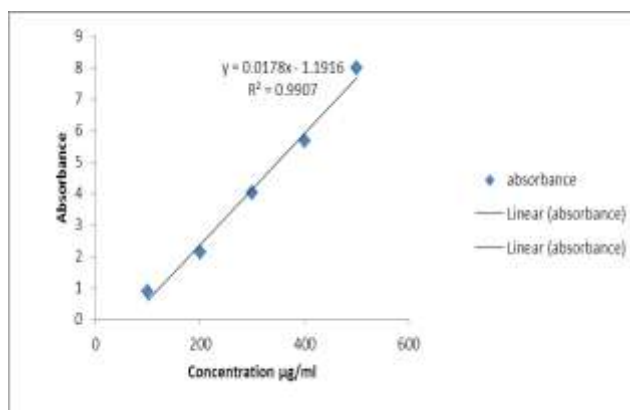


Fig. 1: FRAP assay of Vitamin E.

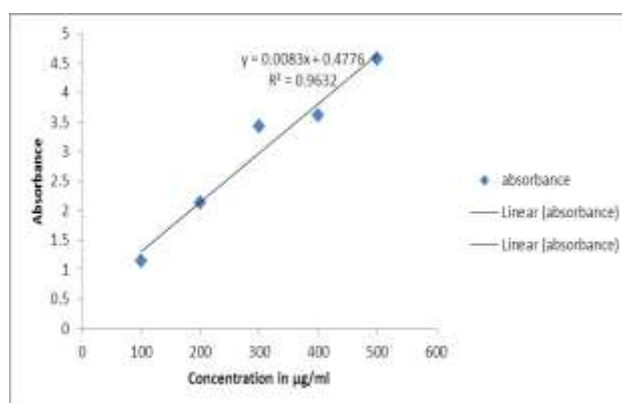


Fig. 2: FRAP assay of essential oil from *A. aitchisonii*.

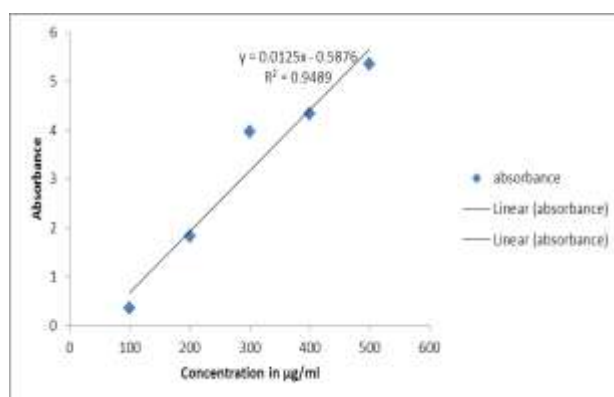


Fig. 3: FRAP assay of ascorbic acid.

4. DISCUSSION

The essential oil from the roots of *A. aitchisonii* has been endowed with diuretic, analgesic, antidiabetic, hepatoprotective, antibacterial, antifungal and antioxidant properties. Our work reveals the antifungal, antibacterial and antioxidant activity of the essential oil from the aerial parts of *A. aitchisonii*. The oil has shown potent effect against *A. tenuis*, *S. sclerotiorum*, *C. graminicola* and *F. oxysporum*. The oil has shown 100% mycelia growth inhibition against

these four fungal strains at a concentration of 2000 μ g/ml, 2000 μ g/ml, 3000 μ g/ml and 3000 μ g/ml respectively. Several synthetic and chemical compounds (fungicides) have been used to preserve and protect food materials and plants from pathogenic fungal infections. However prolonged and increasing use of these substances has led to ecological and environmental hazards which makes it compulsory to adopt new strategies which are less toxic and environmental eco friendly.^[22] The essential oil from *Agrimonia aitchisonii* is found rich in methyl myrtenate,^[7] limonene and camphene.^[23] Flavonoids possess different biological activities like anti-inflammatory, antibacterial, antifungal, antiulcer, anticancer, anti-viral and antispasmodic.^[24] Fungi act as serious pathogens for valuable plants and cause several diseases. Essential oils acts as bio fungicides and have been reported to inhibit postharvest fungi in vitro.^[25] The results of this.

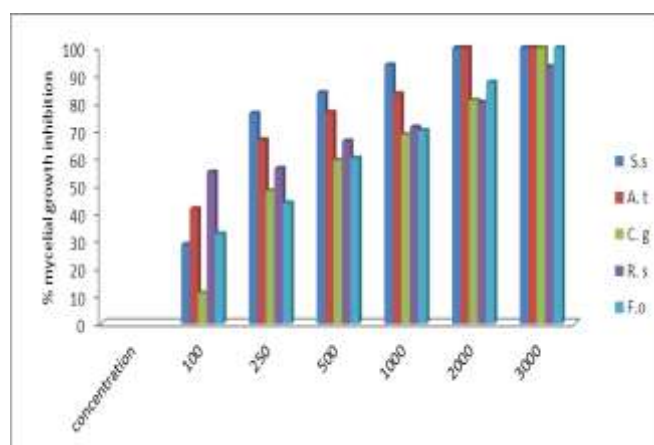


Fig. 4: Effect of essential on the mycelia growth of test fungi at different concentrations. (*S. s* = *S. sclerotiorum*, *A.t* = *A. tenuis*, *C. g* = *C. graminicola*, *R. s* = *R. solani* and *F.o* = *F. oxysporum*).

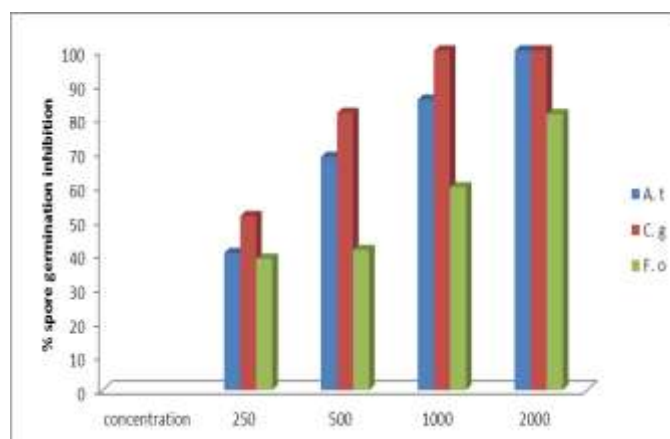


Fig. 5: % spore germination inhibition by essential oil at different concentrations (*A.t* = *A. tenuis*, *C. g* = *C. graminicola*, and *F.o* = *F. oxysporum*).

study showed that essential oil from *Agrimonia aitchisonii* acts as a better, safe and effective bio-fungicide. The essential oil from *A. aitchisonii* showed significant activity against *A. hydrophila* (8mm) and *P. aeruginosa* (7mm) at a concentration of 500µg/ml and lowest effect against *E. coli* and *B. subtilis*. Bacteria acts as serious causative agents for several infections and diseases. The most interesting area of application for medicinal plant extracts is the inhibition of growth and reduction in numbers of the more serious pathogens.^[26,27,28] The ethanolic and aqueous extract of *Agrimonia eupatoria* is reported with antibacterial and wound healing capability^[29], Internally it is used in haematuria and diarrhea, externally for wounds and cuts.^[30] *Staphylococcus aureus* and *pseudomonas aeruginosa* are most common pathogens which infect the skin and *Escherichia coli* which is an opportunistic pathogen at the site of cut wound.^[31] Previous studies shows that the root and seed extracts of *Agrimonia* species reported a small antibacterial activity on *Bacillus cereus*, *Bacillus subtilis* and *Staphylococcus aureus* as well as having some inflammatory and anti diarrheic activity.^[32] A synergistic activity of carvacrol and thymol against some bacteria has been reported.^[33] Polyphenol and Tannins have ability to inactivate the microbial adhesion, enzymes, cell envelope transport protein and thus inhibits the microbial growth.^[34]

FRAP assay measures the reducing potential of an antioxidant reacting with a ferric tripyridyltriazine complex and producing a colored ferrous tripyridyltriazine. Generally, the reducing properties are associated with the presence of phenolics, flavonoids and other compounds which exert their action by breaking the free radical chain by donating hydrogen atom.^[35] The relationship between phytochemicals and antioxidants have been assessing for predicting antioxidant property of the plants, the presence of abundant total phenolics in several medicinal plants exhibited high FRAP values.^[36] Consequently, the essential oil from *A. aitchisonii* results shows exemplary reducing power. In the present study, the trends for ferric ion reducing activities of essential oil from *A. aitchisonii*, clearly increased, due to the formation of the Fe-TPTZ complex with increasing concentration in a dose dependant manner as standard antioxidant ascorbic acid. The essential oil from *A. aitchisonii* showed significant effect against all test fungi and tested strains of bacteria and also significant antioxidant activity. These properties of this oil can contribute towards a substitute for chemical drugs with lesser side effects in comparison to these synthetic medicines and drugs which has leading side effects.

5. CONCLUSION

In this present study, promising antifungal, antioxidant and antibacterial activity has been shown by the essential oil from *A. aitchisonii*. These activities are due to presence of total phenolic, total flavonoid compounds present in the crude oil. Fungi and bacteria act as serious pathogens for valuable plants and animals and cause several diseases. To overcome this problem several synthetic fungicides, drugs and medicines have been developed but these substances leads to several health hazards and environmental pollution so an alternate approach is the need of the hour which includes the development of less hazardous and effective, eco friendly substances. The results of this study suggest that the essential oil of *A. aitchisonii* can be used as an easily accessible source of natural antioxidants, as preservative, as a possible food supplement or in pharmaceutical and substantial source of multipurpose bioactive principles which act as natural antibiotic against various bacterial strains. However, further molecular investigations in order to isolate the antioxidant and bioactive structures actually responsible for this activity need to be carried out.

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