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# Evaluation of some biological activities of different solvent extracted samples of Oat (*Avena Sativa)*

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Plants serve as biological factories of numerous compounds with diverse biological activities. Role of the plants in curing various diseases or infections can be attributed to the presence of such biologically active compounds. The present study focused on the phytochemical screening along with the evaluation of the antimicrobial and phytotoxic potential of *Avena sativa* in various solvent extracts. Ethyl acetate, n-hexane and n-butanol fractions, crude methanolic and aqueous extracts were assessed for their antibacterial potential by disc diffusion method, presence of phytochemical compounds by chemical tests and phytotoxic activity against radish seed. The qualitative phytochemical analyses showed that aqueous and methanol plant extracts had higher concentration of tannins and terpenoids content compared to other solvent extracts. The antimicrobial activity of different extracts against *Xanthomonas campestris, Pseudomonas aeruginosa, Klebsiella pneumonia* and *Candida albicans* revealed 15.17mm, 14.40mm, 14.08mm and 14.43mm zone of inhibition respectively. Furthermore, increase in concentrations of *Avena sativa* extracts and their fractions showed increase in their antimicrobial activity against the tested microbes. The n-butanol fraction reported 17.63mm and 16.42mm zone of inhibition against *Candida albicans* and *Bacillus subtilis*, respectively. For allelopathic activity of crude extract, plant extract negatively affected radish seeds sprouting by showing 83% to 100% inhibition at 100 to 1000 µl/ml concentrations. The *Avena sativa* seed extracts possess biologically active compounds and showed great potential as antimicrobial and phytotoxic agent and therefore may be evaluated for other potential biological activities and applications.

**Keywords: Phytochemical screening, Antimicrobial activities, phytotoxicity, Avena sativa**

## INTRODUCTION

Infectious diseases account for high proportion of health complications in the developing countries including Pakistan. Nowadays situation becomes worse because of the resistance developed by various microorganism against different antibiotic (Roca et al. 2015). This microbial resistance is acquired due to indiscriminate use of commercial antimicrobial drugs commonly used for the treatment of infectious disease. This scenario has forced the researchers to look for new antimicrobial substance from various natural sources including medicinal plants (Amenu, 2014).

Plants play vital role in our lives by providing food, clothes, shelter, ornaments, flavoring and medicine (Castello et al. 2002). Around 60 to 80% of the world's population still depend on drugs that are obtained from plants). Plant extracts have also been exploited for various activities such as antimicrobial, insecticidal, herbicidal, antioxidants, anti-inflammatory, anti-atherosclerotic, anti-tumor, anti-mutagenic, anti-carcinogenic, anti-bacterial and anti-viral activities (Sala et al. 2002, Okpekon et al. 2004, Ogu and Aga, 1995). These secondary metabolites have also been depicted allelopathic activities (Razavi, 2011).

Medicinal plants are of great importance to the health of individuals and communities. The medicinal value of these plants lies in chemical substances that produce a definite physiological action on the human body (Mungole et al. 2010). The most important of these bioactive constituents of plants are alkaloids, tannins, flavonoids, and phenolic compounds (Amin et al. 2013). Primary compounds consist mainly of chlorophyll, protein and common sugar while flavonoid, terpenoids, and phenolic compounds are assigned to the secondary class of phytochemicals (Krishnaiah, 2007). Among the two classes, secondary metabolites have prominent significance medicinal importance (Bhardwaj and Gakhar, 2005).

Aslam et al. (2017) explained allelopathy, referring it to any direct or indirect effect of plants on other plants through the release of chemicals and plays an important role in many agro-ecosystems. Allelochemicals can act on

the stage of germination, growth and development of sensitive plants. The most common changes include the inhibition or retardation of germination, coleoptile elongation, root and shoot development of seedling (Bojovic and Jakovljevic, 2015).

*Avena sativa* (common name: oat), an important Rabi fodder crop, belongs to family *Poacea*. It is a propitious plant with medicinal, nutritional and therapeutic uses (Ben et al. 2015). Green oat extract (above 800 mg dose) has exhibited acute cognitive effects in people of 40-65 years age group (Kennedy et al. 2017). Oat seed extract affects growth inhibition, metal uptake along with scavenging activity of mitochondrial superoxide dismutase. Monodehydroascorbate reductase and dehydroascorbate reductase of oat seeds have great impact on ascorbate regeneration (Vadassery et al. 2009). The present study was mainly focused on phytochemical screening, antimicrobial and allelopathic effects of oats (*Avena Sativa*).

## MATERIALS AND METHODS

### Plant material collection

The plant material used in the current study was collected from local region of Mardan (KPK), Pakistan.

### Preparation of extracts

For sample preparation, seeds of *Avena sativa* were washed using tap water and shade dried before grinding to fine powder form. The fine powder was then soaked in 97% methanol for 7 days at room condition with constant stirring to obtain filtered methanolic crude extract. A part of dried methanolic crude extract (30mg) was dissolved in 300ml distilled water and subjected to fractionation by different solvents. The solution was shifted to separatory funnel and 300ml n-hexane was added to it. After mixing, the solution was allowed to make separate layers of solvents. Upper layer containing n-hexane was collected and lower aqueous layer was again mixed with 300ml n-hexane. The procedure is repeated 3 times for all the solvents (n-hexane, n-butanol and ethyl acetate). After fractionation all the extracts were dried and at the end five different solvent extracts were prepared including methanol, ethyl acetate, n-hexane, water and n-butanol.

### Phyto-chemical analysis

A wide range of Phyto-chemical tests were carried out to detect the presence of various bioactive compounds, including primary metabolites (protein and carbohydrates) and secondary metabolites (terpenoids, flavonoids, saponins, tannins and alkaloids).

### Carbohydrate Test (Fehling's test)

For Carbohydrate determination, 0.1 gm extract of crude extract was dissolved in 5ml of distilled water and filtered. Filtrate was boiled with 1 ml Fehling solution A and 1 ml Fehling solution B. Appearance of red precipitate indicated the presence of reducing sugars.

### Protein Test (biuret test)

The presence of protein in extract was determined by dissolving 0.015 gm of extract in 3ml of distilled water and the solution was filtered. After that, 1 ml $CuSO_4$ solution was added to filtrate. Pink color precipitate formation indicates presence of protein.

### Saponins Test (Frothing test)

Frothing test was conducted to check the presence of saponins. About 0.5 gm plant extract was dissolved in 5ml distilled water. After filtration, $NaHCO_3$ (0.01gm) was added. Persistent froth represented the presence of saponins.

### Tannins Test (Ferric chloride test)

For tannins determination, about 1 to 2 drops of 5% $FeCl_3$ solution were added to filtered extract solution (0.5gm extract and 1ml distilled water). Blue or greenish black color indicated presence of tannins.

### Flavonoids Test

Flavonoids were analyzed using 0.2 gm extract was dissolved in dilute NaOH solution. Few drops of diluted HCl were added to the solution. Resultant colorless solution reported presence of flavonoids in solution.

### Alkaloids Test

For alkaloids, 0.05 gm extract was dissolved in 10ml diluted HCl and filtered. Appearance of white or creamy precipitate upon addition of few drops of Mayer's reagents indicated alkaloids presence.

### Terpenoids Test

Terpenoids were analyzed by dissolving 0.8gm extract in 10ml methanol followed by filtration. About 2ml of sulfuric acid and 1ml of chloroform was added to the filtered extract solution. Reddish brown color of the resultant solution showed the presence of terpenoids.

### Anti-microbial Assay

Nutrient agar media and nutrient broth media were used for the culturing and standardization of different microorganisms respectively. Both media were prepared as described by (Bakht et al. 2011). For media preparation, 2.8 gm nutrient agar media was dissolved in 100 ml distilled water while 3.25 gm nutrient broth media was dissolved in 100 ml distilled water. Prepared media along with all other essential equipment were autoclaved at 121°C for 20 minutes. After pouring media in petri plates, selected microbes along with samples and controls were applied to media plates for assessing antimicrobial activity of samples. The plates were incubated at 37°C for 24 hours and percent (%) growth inhibition was calculated by the following formula:

$$\% \text{ inhibition} = \frac{\text{Zone of inhibition of sample (mm)}}{\text{Zone of inhibition of standard (mm)}} \times 100$$

Where, The positive control (bacteria) = Ciprofloxacin
The positive control (Fungi) = Fluconazole
The negative control = DMSO

**Phytotoxicity Bioassay / Allelopathic Activity**

**Seeds setup**

This test was performed according to the modified protocol of (Mclaughlin et al. 1998). For this, crude extracts (methanolic extract) of the *Avena sativa* plant was integrated at different concentrations (10, 100, 1000 µg/ml) in respective solvents in replicates. Radish seeds were washed with distilled water and then with 1% mercuric chloride. Filter papers were placed in each autoclaved Petri plates. About 5ml of distilled water was added to each control petri plates. Then 5ml of each concentration (10, 100, 1000 µg/ml) was poured in each plate and the respective solvent was allowed to evaporate. 10 radish seeds were placed in control each of the 3 treatment petri plates and spread evenly before the plates were sealed using parafilm, followed by incubation in growth room at 23 ± 2°C for five days. After the seeds were checked and noted daily for five days, after 1 day the seed were checked for sprouting and after 3 days root and shoot measurement (inhibition) was noted. Fresh weight and dry weight were also recorded.

**3.6.2 Seeds observation**

The petri plates were checked on the following day (day 1$^{st}$) for germination or sprouting, and on day 3 and 5 root and shoot lengths were measured.

**3.6.3 Data analysis**

The experimental groups the applied extracts of both plants and control group was compared. i.e. the root and shoot of experimental group were compared with that of control group at day 3$^{rd}$ at the last day 5$^{th}$. And at last the fresh weight and dry weight of all the treated groups were also calculated.

The overall data collected was then organized, tabulated, analyzed and interpreted by using computer, Software MS excel, SPSS v.16 and Statistix 8.1. the significant data (P- value< 0.01) was then further subjected to least significant difference (LSD).

**RESULTS**

**Phytochemical analysis of *Avena sativa* extracts**

The phytochemical screening of different solvent extracted samples of *Avena sativa* revealed the presence and absence of some important secondary metabolites (Table 1). Water and methanol extracts showed presence of all the tested phytochemical constituents i.e. saponins, tannins, alkaloids, flavonoids, terpenoids, carbohydrates and proteins. Whereas n-butanol extract disclosed the presence of tannins, terpenoids, carbohydrates and proteins. Tannins, alkaloids and terpenoids were found in n-hexane while saponins, tannins and carbohydrates were present in ethyl acetate fractions.

The concentration of these phytochemical constituents varied from low and moderate to high in different extracts and fractions. Saponins were present in moderate concentration in methanol extract while low concentration was recorded for water and ethyl acetate fractions. However, tannins were present in high concentration in all the tested extracts except in n-hexane. Furthermore, ethyl acetate, n-butanol and n-hexane fractions showed absence of flavonoids. Whereas they were found in moderate and low concentration in water and methanol extracts respectively. Moderate concentration of alkaloids was detected in methanol extract whereas a low concentration of alkaloids was observed in water and n-hexane fraction. High concentration of terpenoids was detected in water and methanol extracts while its moderate concentration was recorded in n-butanol and water fraction. Moreover, no traces of terpenoids were found in ethyl acetate fraction. Carbohydrates were found in high concentrations in water and n-butanol fractions while resulted in lower concentration in methanol and ethyl acetate and absence in n-hexane fraction. Furthermore, proteins were present in moderate quantities in water and lower concentrations in n-butanol and methanol extracts. No protein was observed in n-hexane and ethyl acetate fraction

**Table 1: List of some Phyto-chemical constituents of different solvent extracted samples of *Avena sativa***

| Solvents | PYTO-CHEMICAL CONSTITUENTS | | | | | | |
|---|---|---|---|---|---|---|---|
| | Saponins | Tannins | Alkaloids | Flavonoids | Terpenoids | Carbohydrates | Proteins |
| **Water** | + | +++ | + | ++ | +++ | +++ | ++ |
| **Methanol** | ++ | +++ | ++ | + | +++ | + | + |
| **Butanol** | _ | +++ | _ | _ | ++ | +++ | + |
| **N-Hexane** | _ | + | + | _ | ++ | _ | _ |
| **Ethyl-Acetate** | + | +++ | | _ | _ | + | _ |

Legend: += Low concentration, ++= Moderate concentration, +++= High Concentration, - = Absent

**Antimicrobial activities**

All the five test samples of *Avena sativa* seeds extracts were screened for their antimicrobial properties against seven (7) microbes at three different (0.5mg, 1mg and 1.5mg) concentrations. Ciprofloxacin was used as positive control in case of bacteria and fluconazole in case of fungi while DMSO was used as negative control during experiment. Statistically, significance (p-value < 0.01) of the results was calculated for solvent systems, concentrations of samples and for the interaction between them. Moreover, increase in Zone of Inhibition (ZOI) was carried out with increase in sample concentration during experiment.

Table 2 represents the growth inhibition of *E. coli* carried out by different test samples. Results indicated that different ZOI were measured for different test samples against *E. coli* ranging from 8.43 mm to 14.53mm. Among all the test samples, the highest ZOI of 14.53 mm was recorded for n-butanol fraction followed by 13.63mm ZOI noted for n-hexane and 13.53mm ZOI for ethyl acetate all at 1.5mg sample concentration. The least ZOI (8.43mm) was observed for n-butanol at 0.5mg concentration

According to the mean of overall dosage application of different solvent extracts and fractions the highest zone of inhibition was observed for *Avena sativa* seeds Ethyl-acetate fraction (12.23 mm) followed by n-butanol (11.90 mm), methanol (11.64 mm) and n-hexane (11.31 mm). The least zone of inhibition among the samples was observed for water fraction with mean value of 10.02 mm against *E. coli*.

Statistical analysis also revealed that different concentrations of samples carried out corresponding growth inhibition of microbes. Among all the samples that inhibited microbial growth, highest sample concentration reported the maximum growth reduction. The maximum mean zone of inhibition i.e. 12.91 mm was recorded at highest concentration (1.5mg), followed by 1 mg dose i.e. 11.88 mm and the least ZOI 9.47 mm was noted for minimum concentration (0.5 mg) applied.

Table 3 indicates the zone of inhibition of various extracts and fractions of *Avena sativa* seeds at three different concentrations against *Bacillus subtilis*. Results showed that all the samples applied at all three doses showed antibacterial activity and Zone of Inhibition (ZOI) were measured in the range of 8.87 mm to 18 mm, which was lower than zone of inhibition (33.14 mm) of positive control (Ciprofloxacin) against *Bacillus subtilis*. Among all the samples, the highest zone of inhibition (18 mm) was observed for n-hexane fraction when applied at 1.5 mg concentration followed by methanol extract (16.30 mm) applied at 1.5 mg. The minimum ZOI was recorded for n-butanol (8.87 mm) fraction at 0.5 mg concentration.

Among the solvents used in experiment, the highest mean ZOI was calculated for n-hexane fraction (16.42 mm) followed by methanol extract (13.34 mm) and ethyl acetate (12.46 mm). The least mean ZOI was recorded for water fraction (11.98 mm). Upon interaction of solvent system, the mean of highest dose application reported the highest antibacterial activity against *Bacillus subtilis* i.e. 15.27 mm, followed by 1 mg and then the least at 0.5 mg dose with 12.47 and 9.63 mm ZOI, respectively.

**Table 2: Effect of *Avena sativa* extracts on *Escherichia coli***

| Solvent System | Concentration per disc | | | Mean | Ciprofloxacin (mm) | DMSO |
|---|---|---|---|---|---|---|
| | 0.5 mg | 1 mg | 1.5 mg | | | |
| **Water** | 9.43 | 10.10 | 10.53 | 10.02 B | 33.14 | 0 |
| **Methanol** | 10.53 | 12.07 | 12.33 | 11.64 AB | | |
| **n-butanol** | 8.43 | 12.73 | 14.53 | 11.90 A | | |
| **n-hexane** | 8.87 | 11.43 | 13.63 | 11.31 AB | | |
| **Ethyl-acetate** | 10.10 | 13.07 | 13.53 | 12.23 A | | |
| **Mean** | 9.47 C | 11.88 B | 12.91 A | | | |

Means followed by different letter (s) are significantly different from each other (P < 0.05)

LSD value for solvent system = 0.88,LSD value for doses = 0.48 ,LSD value for interaction of solvent system and doses = 0.53

**Table 3: Effect of *Avena sativa* extracts on *Bacillus subtilis***

| Solvent System | Concentration per disc | | | Mean | Ciprofloxacin (mm) | DMSO |
|---|---|---|---|---|---|---|
| | 0.5 mg | 1 mg | 1.5 mg | | | |
| Water | 9.63 | 12.20 | 14.10 | 11.98 B | 33.14 | 0 |
| Methanol | 10.87 | 12.87 | 16.30 | 13.34 B | | |
| Butanol | 8.87 | 13.33 | 14.63 | 12.28 B | | |
| n-hexane | 15.63 | 15.63 | 18.00 | 16.42 A | | |
| Ethyl-acetate | 9.63 | 12.47 | 15.27 | 12.46 B | | |
| Mean | 10.93 C | 13.30 B | 15.66 A | | | |

Means followed by different letter (s) are significantly different from each other (P < 0.05)

LSD value for solvent system = 1.14, LSD value for doses = 0.76

LSD value for interaction of solvent system and doses = 0.62

Results of antibacterial activity of different test samples at three different concentrations against *Xanthomonas campestris* is represented in table 4. The zone of inhibition (ZOI) of all the samples against the microbe were in range of 8.97 mm to 16.63 mm. Among all the solvent systems, n-hexane fraction was most potent against microbe and reported the highest antibacterial activity followed by ethyl acetate, water, n-butanol and the least methanol with 16.63, 16.40, 16.20, 15.30 and 14.97 mm zone of inhibition, respectively at the highest tested concentration of 1.5 mg sample. The highest mean ZOI among all the tested samples was observed for ethyl acetate (15.17mm) followed by n-hexane (15.08 mm), n-butanol (13.90 mm), methanol (12.59 mm). The minimum mean ZOI (12.38 mm) was recorded for water fraction. The minimum dose (0.5 mg) reported least mean antibacterial activity against *Xanthomonas campestris* (11.27 mm ZOI) while higher concentrations 1mg and 1.5 mg reported higher ZOI of 14.29 mm and 15.90 mm, respectively.

Table 5 indicates the antibacterial effects of five different *Avena sativa* seed extracts and fractions at three different concentrations against *Staphylococcus aureus.* Data reveals that all the different solvent extracted samples applied at three different concentrations reduced growth of the microbe by showing zone of inhibition (ZOI) in the range of 8.63 mm to 15.97 mm. Among all the samples, the maximum ZOI was observed for n-butanol fraction (15.97 mm) at 1.5 mg sample followed by methanol extract (15.63 mm). Whereas the least ZOI was observed for water fraction (8.63 mm) applied at minimum concentration of 0.5 mg.

Among the solvent systems, the mean highest antibacterial activity was calculated for n-hexane plant seed extract (12.70 mm) followed by n-butanol fraction (12.59 mm) and ethyl acetate fraction (12.19 mm). The least mean ZOI (10.89 mm) was recorded for water fraction. Interaction to solvent system, the mean highest concentration application carried out the highest antibacterial activity (14.89 mm ZOI) against *Staphylococcus aureus* followed by 12.14 mm ZOI at 1 mg while the least 9.28 mm ZOI at 0.5 mg concentration against the same microbe.

**Table 4: Effect of *Avena sativa* extracts on *Xanthomonas campestris***

| Solvent system | Concentration per disc | | | Mean | Ciprofloxacin (mm) | DMSO |
|---|---|---|---|---|---|---|
| | 0.5 mg | 1 mg | 1.5 mg | | | |
| Water | 8.97 | 11.97 | 16.20 | 12.38 B | 33.14 | 0 |
| Methanol | 8.97 | 13.83 | 14.97 | 12.59 B | | |
| Butanol | 11.30 | 15.10 | 15.30 | 13.90 AB | | |
| n-hexane | 12.97 | 15.63 | 16.63 | 15.08 A | | |
| Ethyl-acetate | 14.17 | 14.93 | 16.40 | 15.17 A | | |
| Mean | 11.27 C | 14.29 B | 15.90 A | | | |

Means followed by different letter (s) are significantly different from each other ($P < 0.05$)
LSD value for solvent system = 1.14
LSD value for doses = 0.65
LSD value for interaction of solvent system and doses = 0.64

**Table 5: Effect of *Avena sativa* extracts on *Staphylococcus aureus***

| Solvent System | Concentration per disc | | | Mean | Ciprofloxacin (mm) | DMSO |
|---|---|---|---|---|---|---|
| | 0.5 | 1 | 1.5 | | | |
| Water | 8.63 | 11.30 | 12.73 | 10.89 A | 33.14 | 0 |
| Methanol | 8.87 | 11.97 | 15.63 | 12.16 A | | |
| Butanol | 9.63 | 12.17 | 15.97 | 12.59 A | | |
| n-hexane | 9.63 | 13.40 | 15.07 | 12.70 A | | |
| Ethyl-acetate | 9.63 | 11.87 | 15.07 | 12.19 A | | |
| Mean | 9.28 C | 12.14 B | 14.89 A | | | |

LSD value for solvent system = 1.20
LSD value for doses = 0.36
LSD value for interaction of solvent system and doses = 0.37

The results showing antibacterial potential of different solvent extracted samples of *Avena sativa* seeds at three different concentrations against *Pseudomonas aeruginosa* is given in table 6. Different samples showed different ZOI against the microbe ranging from 8.43mm to 16.43 mm. Among all the samples, maximum ZOI was observed for n-butanol fraction (16.43 mm) followed by ethyl acetate fraction (16.27 mm) and n-hexane fraction (12.87mm) applied at 1.5mg concentration dose. The minimum ZOI among the various solvent extracts was observed for n-hexane fraction (8.43 mm) applied at the least test concentration of 0.5 mg.

For the mean of overall dosage application of different solvent system extracts, the highest ZOI was observed for n-butanol (14.94 mm) followed by ethyl acetate (14.40 mm), methanol (11.01 mm) and n-hexane (10.34 mm) fraction. The least ZOI among the solvents used was observed for water fraction of seeds extract that was recorded with a mean of 10.04 mm of inhibition zone against *Pseudomonas aeruginosa*.

Accordingly, the highest mean ZOI (13.88 mm) was recorded for maximum concentration (1.5mg) used in experiment followed by 12.19 mm ZOI for 1mg concentration and the least ZOI (10.38 mm) was observed for 0.5mg concentration.

Table 7 indicates the effects of five different solvent extracted samples of *Avena sativa* seeds on growth of *Klebsiella pneumoniae* at 3 different concentrations**.** The ZOI measured for all the samples were in the range of 8.63mm to 17.10 mm.

The highest zones of inhibition were observed for the maximum applied concentration (1.5mg) during experiment. Among all the solvents used, ethyl acetate extract showed the highest antibacterial activity followed by water, methanol, n-butanol and n-hexane fraction with 14.08, 13.19, 12.64, 11.47- and 11.44-mm zone of inhibition, respectively. The highest mean zone of inhibition among the solvent system extract was observed for ethyl acetate(14.08mm) followed by water (13.19mm), methanol (12.64mm), Butanol (11.47mm) and the least N-hexane plant seed extract (11.44mm) As for the concentrations, the least mean antibacterial activity (9.43 mm ZOI) was recorded for 0.5 mg concentration, and ZOI was increased up to 12.75 and 15.52 mm for 1 and 1.5 mg concentration of samples against *Klebsiella pneumonia*.

Table 8 indicates the observed zone of inhibition for various samples of *Avena sativa* seeds extracts at three different concentrations against *Candida albicans*. Results showed that all the solvent extract and fractions applied at all three concentrations showed antifungal activity, ranging from 7 mm to 20.73 mm ZOI. The highest zone of inhibition was reported by n-hexane (20.73 mm) fraction followed by methanol extract (16.53 mm) and ethyl acetate (15.73mg) when applied at 1.5 mg concentration. The least zone of inhibition was recorded for water fraction (7 mm) at 0.5 mg concentration. Among the solvent systems, the highest mean zone of inhibition was calculated for n-hexane fraction (17.63 mm) followed by methanol extract (15.01 mm) and ethyl acetate (14.43mm). The least mean ZOI was recorded for water (9.09 mm) fraction. For interaction to solvent system, the mean of highest concentration application was observed with the highest antifungal activity against *Candida albicans* i.e. 15.83 mm ZOI for 1.5mg concentration, followed by 13.75mm ZOI for 1 mg concentration and 12.68 mm ZOI at 0.5 mg sample concentration, respectively.

**Table 6: Effect of *Avena sativa* extracts on *Pseudomonas aeruginosa*.**

| Solvent System | Concentration per disc | | | Mean | Ciprofloxacin (mm) | DMSO |
|---|---|---|---|---|---|---|
| | 0.5 mg | 1 mg | 1.5 mg | | | |
| **Water** | 8.63 | 9.63 | 11.87 | 10.04 B | | |
| **Methanol** | 9.43 | 11.63 | 11.97 | 11.01 B | | |
| **Butanol** | 13.20 | 15.20 | 16.43 | 14.94 A | | |
| **n-hexane** | 8.43 | 9.73 | 12.87 | 10.34 B | 33.14 | 0 |
| **Ethyl-acetate** | 12.20 | 14.73 | 16.27 | 14.40 A | | |
| **Mean** | 10.38 B | 12.19 A | 13.88 A | | | |

Means followed by different letter (s) are significantly different from each other (P < 0.05)
LSD value for solvent system = 0.84 ,LSD value for doses = 0.86

**Table 7: Effect of *Avena sativa* extracts on *Klebsiella pneumonia***

| Solvent System | Concentration per disc | | | Mean | Ciprofloxacin (mm) | DMSO |
|---|---|---|---|---|---|---|
| | 0.5 mg | 1 mg | 1.5 mg | | | |
| **Water** | 8.63 | 13.83 | 17.10 | 13.19 A | | |
| **Methanol** | 8.63 | 13.10 | 16.20 | 12.64 A | | |
| **Butanol** | 8.50 | 11.07 | 14.83 | 11.47 A | | |
| **n-hexane** | 9.53 | 11.63 | 13.17 | 11.44 A | | |
| **Ethyl-acetate** | 11.83 | 14.10 | 16.30 | 14.08 A | 33.14 | 0 |
| **Mean** | 9.43 B | 12.75 A | 15.52 A | | | |

Means followed by different letter (s) are significantly different from each other (P < 0.05)
LSD value for solvent system = 1.34,LSD value for doses = 0.86,LSD value for interaction of solvent system and doses = 0.50

**Table 8: Effect of *Avena sativa* extracts on candida albicans**

| Solvent Systems | Concentration per disc | | | Mean | Fluconazole (mm) | DMSO |
|---|---|---|---|---|---|---|
| | 0.5 mg` | 1mg | 1.5 mg | | | |
| **Water** | 7.00 | 9.63 | 10.63 | 9.09 C | | |
| **Methanol** | 13.30 | 15.20 | 16.53 | 15.01 B | | |
| **Butanol** | 14.07 | 13.20 | 15.53 | 14.27 B | | |
| **n-hexane** | 15.30 | 16.87 | 20.73 | 17.63 A | 22 | 0 |
| **Ethyl-acetate** | 13.73 | 13.83 | 15.73 | 14.43 B | | |
| **Mean** | 12.68 B | 13.75 AB | 15.83 A | | | |

Means followed by different letter (s) are significantly different from each other (P < 0.05)
LSD value for solvent system = 0.93,LSD value for doses = 1.16

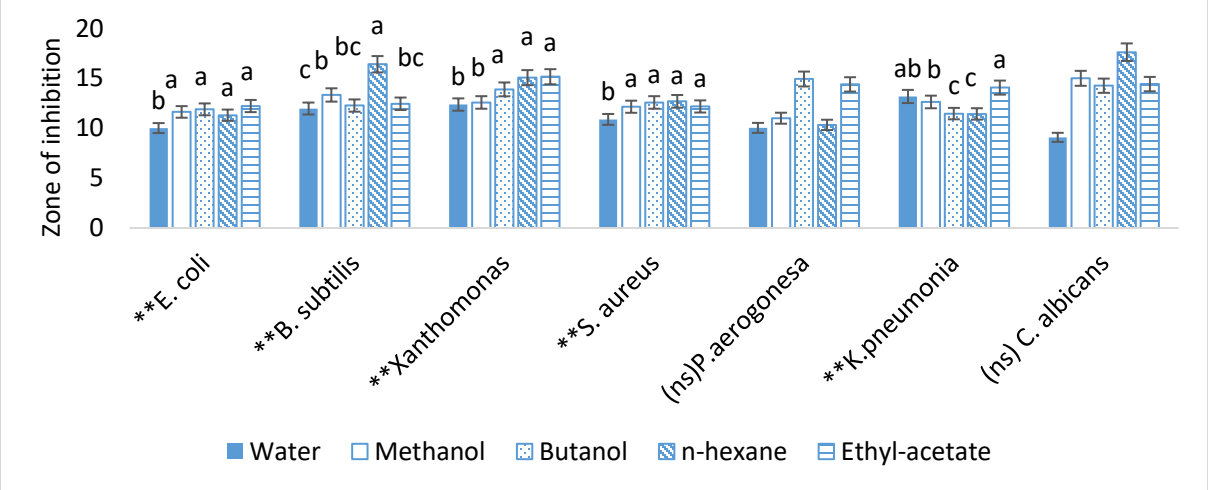


**Figure 1: Antimicrobial activities of various solvent system *Avena sativa* seed extract against different microbes**

****, significant; ns, non-significant**

The effects of different solvent extracted samples on various microbes is given in figure 1. The highest antimicrobial efficacy was illustrated by n-hexane fraction against *Candida albicans* (17.63 mm) fungi, followed by *Bacillus subtilis* (16.42 mm) bacteria. The least antimicrobial efficacy was recorded for water fraction (9.09 mm ZOI) against *Candida albicans* followed by *E. coli* and then *Pseudomonas aeruginosa* with 10.02 and 10.04 mm zone of inhibition, respectively. Moreover, the ZOI measured for different samples against the tested microbes varied and was in range of 10.34mm to 15.17 mm.

Figure 2 represents the mean effect of different concentrations of all the solvent extracted samples on the test microbes. Results clearly illustrates that with increase in concentration, the zone of inhibition of all the microbes increased.

**Phytotoxic activity of *Avena sativa***

The phytotoxic activity of the plant extract against radish seeds from day 1 to day 5, and the weight (fresh and dry) of the radish seeds placed in distill water along with different level of plant extract is shown in table 4.9. Results showed that after first day, the seeds sprouting was observed maximum with distill water (86.6 %) compared to other seed germination medium (distill water) with different level of plant extract.

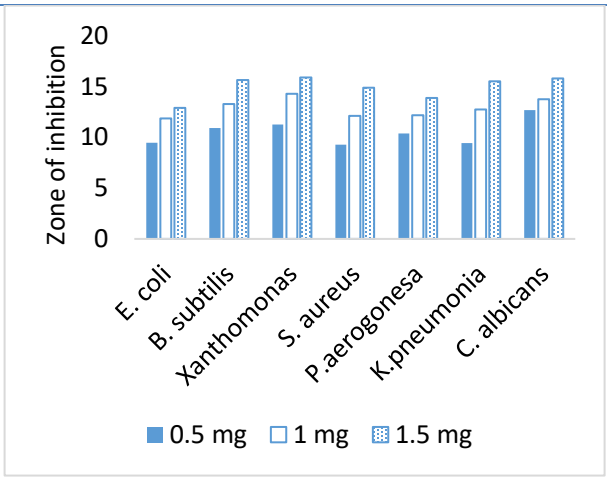


**Figure 2: The effect of different doses of plant mean extract against various microbes**

The other medium used for radish seed germination with addition of different level of plant extract had shown inversely relation of sprouting seeds with plant extract quantity, with highest sprouting of seeds (23.3 %) with 10 ul/ml plant extract, followed by 100 ul/ml (20 %) and least sprouting seeds in 1000 ul/ml (3.3 %) plant extract germination medium. After day 3, the plantlets produced had shown maximum root (1.56 cm) and shoot length (0.45 cm) in distill water only compare to other medium with supplement of different level of plant extract. Among the different level of plant extracts medium, the maximum root length was observed the least plant extract supplement i.e. 10 ul/ml (0.23 cm) followed by 100 ul/ml (0.21 cm), whereas in 1000 ul/ml plant extract medium had shown complete inhibition of the radish root. On the other hand, the shoot length on day 3 had been observed maximum for 100 ul/ml (0.44 cm) followed by 10 ul/ml (0.34 cm) and the least in radish medium with supplement of 1000 ul/ml of plant extract (0.1 cm). After day 5, the root length of germinated radish plant had shown maximum root and shoot length with measurement of 3.81 and 1.51 cm, respectively. The response of plantlets recorded for 10, 100 and 1000 ul/ml plant extract medium of radish plant after day 5 showed maximum root and shoot length in 100 ul/ml (0.26 and 0.52 cm) followed by 10 ul/ml (0.23 and 0.37 cm) and least with 1000 ul/ml plant extract medium for shoot length (0.1 cm), however no measurement was recorded for root length at this plant concentration (1000 ul/ml).

**Table9: Phytotoxic activity of Avena sativa extract against Radish plant**

| Days | Parameters | 0 µl/ml | 10µl/ml | 100µl/ml | 1000µl/ml |
|---|---|---|---|---|---|
| Day 1 | Sprouting of seeds (%) | 86.6 ±11.4 | 23.3 ±11.54 | 20 ±10 | 3.3 ±5.7 |
| Day 3 | Root length (cm) | 1.56 ±0.707 | 0.23 ±0.4 | 0.21 ±0.36 | 0.00 ±0.00 |
| | Shoot length (cm) | 0.45 ±0.161 | 0.34 ±1.21 | 0.44 ±0.21 | 0.1 ±0.17 |
| Day 5 | Root length (cm) | 3.81 ±1.60 | 0.23 ±0.07 | 0.26 ±0.37 | 0.00 ±0.00 |
| | Shoot length (cm) | 1.51 ±0.65 | 0.37 ±0.4 | 0.52 ±0.39 | 0.1 ±0.17 |

**(p-value < 0.01)**

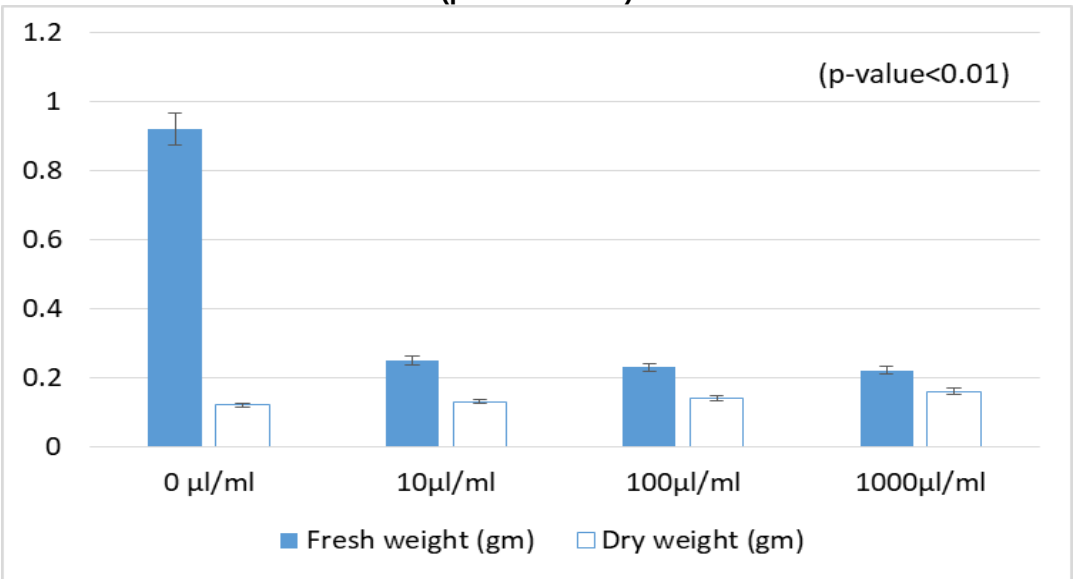


**Figure 4.3: Fresh/dry weight of radish plantlets at different time interval.**

The fresh weight determine of the radish plant was maximum for plantlets in 0 µl/ml (0.92 gm) medium only as shown in figure 4.3. Among the different level of plant extracts, the highest fresh weight was observed with 10 ul/ml plant extract medium with resultant 0.25 gm, followed by 100 ul/ml (0.23 gm) and then the least fresh weight of radish plant germinated on 1000 ul/ml plant extract medium (0.22 gm) was observed. The dry weight of the radish plant germinated on 0 µl/ml was recorded with 0.12 gm, whereas at 10, 100 and 1000 ul/ml, the resultant dry weight was observed 0.13, 0.14 and 0.16 gm, respectively.

## DISCUSSION

The phytochemical screening of different extracts of *Avena sativa* showed presence of tannins, flavonoids, carbohydrates, alkaloids, proteins, terpenoids and saponins. The concentration and availability of these constituents varied among the various solvent extracts including methanol, n-hexane, water and ethyl acetate extract. Such secondary metabolites have the potential against a broad spectrum of organisms Usha et al. (2014) reported presence of carbohydrates using Hling, Benedict, Barfoed and Molisch test, alkaloids by Mayer, Wagner, Hager's and Dragendorff test, steroids through Libermann Burchard test and salkowski test, flavonoids by Shinoda test and saponins by foam test in *Avena sativa* seed methanolic extract. Furthermore, tannins and triterpinoids were not found. Kaur et al. (2014) reported presence of saponins and alkaloids and absence of phytosterol and glycosides in hydroalcoholic and petroleum ether extracts of *Avena sativa.* Carbohydrates in form of glucose, pentosans, mucilage (b-glucan), sucrose, neokestose, neobifurcose, acid galactoarabinoxylan, cellulose, ketose, bifurcose and fructose are present in *Avena sativa* (Franz, 1994, Wood, 2007, Truswell, 2002). The Oat is only cereal that is rich in avenalin (a globulin protein), comprising of 80 % of storage protein (Brinegar and Peterson, 1982). Oat's hull-less kernel has highest protein content among all cereals. Gramine, Oat's indole alkaloid, exhibit weak sedative effects (Duke and Duke, 2018). Avenanthramides has been isolated from *Avena sativa* methanolic extract ranges from 2-53 mg per kg (Collins,1989, Tsetsegmaa and Tsetsegee, 2012, Chen et al. 2007) reported 28 different flavonoids in green parts and seeds of oat and further supports the present research findings. The flavonoids act as protective agent against some major cereal crop nematodes and may have phytoalexin properties (Soriano et al. 2004) Avaenacin saponin play role in disease resistance cultivars development (Kitchen et al. 2003) also determined low concentrations of tannins and saponin, moderate concentration of flavonoids and protein and higher concentrations of carbohydrates in oat and strengthens our findings.

According to Singh and Belkheir, (2013) different phytochemicals of oat may have neurotonic, diuretic, antioxidant, antidiabetic, phytotoxic, stimulant, anticholesterolemic, antitumor, antispasmodic and anti-inflammatory properties. In the present study various solvent extracts of oat including methanol, n-hexane, aqueous, ethyl acetate and n-butanol extracts showed different antimicrobial potential against *P. aeruginosa, E. coli, S. aureus, K. pneumonia, X. campestris, B. subtilis and C. albicans*. (Sørensen et al. 2011) reported antifungal activity of oat seed proteins against *Penicillium roqueforti* and described that antifungal activity of specific phytochemical of the class I chitinase was 10 times higher than wheat, barley and rye. Mukherjee et al. (2012) evaluated antibacterial activity of *Alstonia scholaris* n-hexane fraction and reported remarkable activity of the sample which supports our results by affirming that n-hexane extract contains antibacterial potential against *Serratia marcescens* and *Enterobacter cloacae* members of Enterobacteriaceae. Results of present study are also supported by the findings of (Weerakkody et al. 2010). The researchers observed higher antibacterial activity of n-hexane extract than water and ethanol extracts against *S. typhimurium, L. monocytogens, E. coli* and *S. aureus.*

The *Avena sativa* extracts also revealed phytotoxic activity by inhibiting shoot and root growth ultimately affecting fresh and dry weights of plants. John and Sarada, (2012) reported that saponin of *Avena sativa* had no phytotoxic potential while flavonoids of same plant affected germination and seedling growth up to 96%. Length of root from shoot was found sensitive during present study and similar sensitivity was determined by (Kato-Noguchi and Morokuma, 2007) also reported more sensitivity of root than hypocotyl length.

## CONCLUSION

Based on these results, it can be concluded that different phytochemicals including tannins, terpenoids, saponins, flavonoids, carbohydrates, proteins and alkaloids were present in different extracts of *Avena sativa* in different concentrations. Among all the samples, n-hexane and n-butanol extracts showed significant antimicrobial activity against test microbes. The tested extracts also reported significant phytotoxic activity against sprouting of radish seeds and growth of root and shoot of seedlings.

## CONFLICT OF INTEREST

The authors declared no conflict of interest.

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## AUTHOR CONTRIBUTIONS

MA conceptualization. FS and BA Methodology, performance of experiments and manuscript writing. AR data curation and formal analysis. ABR and MU Writing – review & editing. All authors read and approved the final

version.



## REFERENCES

Amenu D (2014) *Antimicrobial Activity of Medicinal Plant Extracts and Their Synergistic Effect on Some Selected Pathogens*.

Amin Mir M, Sawhney S, Jassal M (2013) 2013 Wudpecker Journals Qualitative and quantitative analysis of phytochemicals of Taraxacum officinale. *Wudpecker J Pharm Pharmocology*.

Aslam F, Khaliq A, Matloob A, Tanveer A, Hussain S, Zahir ZA (2017) Allelopathy in agro-ecosystems: a critical review of wheat allelopathy-concepts and implications. *Chemoecology*. doi:10.1007/s00049-016-0225-x.

Bakht J, Tayyab M, Ali H, Islam A, Shafi M (2011) Effect of different solvent extracted sample of Allium sativum (Linn) on bacteria and fungi. *African J Biotechnol*. doi:10.5897/ajb11.232.

Ben Halima N, Ben Saad R, Khemakhem B, Fendri I, Abdelkafi S (2015) Oat (Avena sativa l.): Oil and nutriment compounds valorization for potential use in industrial applications. *J Oleo Sci*. doi:10.5650/jos. ess15074.

Bhardwaj S, Gakhar S (2005) Ethnomedicinal plants used by the tribals of Mizoram to cure cuts & wounds. *Indian J Tradit Knowl*.

Bojovic B, Jakovljevic D (2015) Allelopathic relations of selected cereal and vegetable species during seed germination and seedling growth. *Kragujev J Sci*. doi:10.5937/kgjsci1537135b.

Brinegar AC, Peterson DM (1982) Separation and characterization of oat globulin polypeptides. *Arch Biochem Biophys*. doi:10.1016/0003-9861(82)90135-7.

Castello MC, Phatak A, Chandra N, Sharon M (2002) Antimicrobial activity of crude extracts from plant parts and corresponding calli of Bixa orellana L. *Indian J Exp Biol*.

Chen CYO, Milbury PE, Collins FW, Blumberg JB (2007) Avenanthramides are bioavailable and have antioxidant activity in humans after acute consumption of an enriched mixture from oats. *J Nutr*. doi:10.1093/jn/137.6.1375.

Collins FW (1989) Oat Phenolics: Avenanthramides, Novel Substituted N-Cinnamoylanthranilate Alkaloids from Oat Groats and Hulls. *J Agric Food Chem*. doi:10.1021/jf00085a015.

Duke JA, Duke JA (2018) Handbook of Phytochemical Constituents of GRAS Herbs and Other Economic Plants. In: *Handbook of Phytochemical Constituents of GRAS Herbs and Other Economic Plants* doi:10.1201/9780203752623-1.

Franz G (1994) Hagers Handbuch der Pharmazeutischen Praxis, Band IV, Drogen A - D, Herausgeber: R. Hänsel, K. Keller, H. Rimpler, G. Schneider, Springer-Verlag, 1992; DM 750,—, Subskriptionspreis DM 600,—. *Arch Pharm (Weinheim)*. doi:10.1002/ardp.19943270315.

John J, Sarada S (2012) Role of phenolics in allelopathic interactions. *Allelopath J*.

Kato-Noguchi H, Morokuma M (2007) Ethanolic fermentation and anoxia tolerance in four rice cultivars. *J Plant Physiol*. doi:10.1016/j.jplph.2005.09.017.

Kaur D, Kamboj A, Shri R, Gujral IK (2016) comparative evaluation of anxiolytic effects of various extracts of oats (avena sativa), rice bran (oryza sativa) and spinach (spinacia oleracea) in experimental animals. *Int J Pharm Sci Res*. doi:10.13040/IJPSR.0975-8232.7(10).4110-16.

Kennedy DO, Jackson PA, Forster J, Khan J, Grothe T, Perrinjaquet-Moccetti T, Haskell-Ramsay CF (2017) Acute effects of a wild green-oat (Avena sativa) extract on cognitive function in middle-aged adults: A double-blind, placebo-controlled, within-subjects trial. *Nutr Neurosci*. doi:10.1080/1028415X.2015.1101304.

Kitchen JL, McDonald GK, Shepherd KW, Lorimer MF, Graham RD (2003) Comparing wheat grown in South Australian organic and conventional farming systems. 1. Growth and grain yield. *Aust J Agric Res*. doi:10.1071/AR03039.

Krishnaiah Bono DRSA (2007) Phytochemical antioxidants for health and medicine - A move towards nature. *Biotechnol Mol Biol rfeview Vol 1 pp097-104, Sept 2007*.

Mclaughlin JL, Rogers LL, Anderson JE (1998) The use of biological assays to evaluate botanicals. *Ther Innov Regul Sci*. doi:10.1177/009286159803200223.

Mukherjee S, Sushma V, Patra S, Barui AK, Bhadra MP, Sreedhar B, Patra CR (2012) Green chemistry approach for the synthesis and stabilization of biocompatible gold nanoparticles and their potential applications in cancer therapy. *Nanotechnology*. doi:10.1088/0957-4484/23/45/455103.

Mungole A, Day S, Kamble R, Kanfade H, Chaturvedi A, Zanwar P (2010) Active phytochemical and antibacterial potentiality of in-vitro regenerated plantlets of Canscora decurrens (Dalzell). *Indian J Sci Technol*. doi:10.17485/ijst/2010/v3i6/29782.

Ogu EO, Agu RC (1995) A comparison of some chemical properties of Garcinia kola and hops for assessment

of Garcinia brewing value. *Bioresour Technol*. doi:10.1016/0960-8524(95)00081-X.

Okpekon T, Yolou S, Gleye C, Roblot F, Loiseau P, Bories C, Grellier P, Frappier F, et al (2004) Antiparasitic activities of medicinal plants used in Ivory Coast. *J Ethnopharmacol*. doi:10.1016/j.jep.2003.09.029.

Osbourn AE (2003) Saponins in cereals. *Phytochemistry*. doi:10.1016/S0031-9422(02)00393-X.

Razavi SM (2011) Plant coumarins as allelopathic agents. *Int J Biol Chem*. doi:10.3923/ijbc.2011.86.90.

Roca I, Akova M, Baquero F, Carlet J, Cavaleri M, Coenen S, Cohen J, Findlay D, et al (2015) Corrigendum to The global threat of antimicrobial resistance: Science for intervention [New Microbes New Infect, 6, (2015), 22-29] Doi: 10.1016/j.nmni.2015.02.007. *New Microbes New Infect*. doi:10.1016/j.nmni.2015.09.004.

Sala A, Recio M del C, Giner RM, Máñez S, Tournier H, Schinella G, Ríos J-L (2002) Anti-inflammatory and antioxidant properties of Helichrysum italicum . *J Pharm Pharmacol*. doi:10.1211/0022357021778600.

Singh R, De S, Belkheir A (2013) Avena sativa (Oat), A Potential Neutraceutical and Therapeutic Agent: An Overview. *Crit Rev Food Sci Nutr*. doi:10.1080/10408398.2010.526725.

Sørensen HP, Madsen LS, Petersen J, Andersen JT, Hansen AM, Beck HC (2010) Oat (Avena sativa) seed extract as an antifungal food preservative through the catalytic activity of a highly abundant class i chitinase. *Appl Biochem Biotechnol*. doi:10.1007/s12010-009-8557-4.

Soriano IR, Asenstorfer RE, Schmidt O, Riley IT (2004) Inducible flavone in oats (Avena sativa) is a novel defense against plant-parasitic nematodes. *Phytopathology*. doi:10.1094/PHYTO.2004.94.11.1207.

The Chemistry of Cereal Proteins (2017) doi:10.1201/9780203737057.

Truswell AS (2002) Cereal grains and coronary heart disease. *Eur J Clin Nutr*. doi:10.1038/sj.ejcn.1601283.

Tsetsegmaa R, Tsetsegee D (2012) Technological study of an oat-based fermented product. In: *Proceedings - 2012 7th International Forum on Strategic Technology, IFOST 2012* doi:10.1109/IFOST.2012.6357514.

Usha Rani K, Ramaiah M, Nagaphani K, Preethi V, Srinadh M (2014) Screening for antidepressant-like effect of methanolic seed extract of Avena sativa using animal models. *Pharmacogn J*. doi:10.5530/pj.2014.3.13.

Vadassery J, Tripathi S, Prasad R, Varma A, Oelmüller R (2009) Monodehydroascorbate reductase 2 and dehydroascorbate reductase 5 are crucial for a mutualistic interaction between Piriformospora indica and Arabidopsis. *J Plant Physiol*. doi:10.1016/j.jplph.2008.12.016.

Weerakkody NS, Caffin N, Turner MS, Dykes GA (2010) In vitro antimicrobial activity of less-utilized spice and herb extracts against selected food-borne bacteria. *Food Control*. doi:10.1016/j.foodcont.2010.04.014.

Wood PJ (2007) Cereal β-glucans in diet and health. *J Cereal Sci*. doi:10.1016/j.jcs.2007.06.012.