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## EXPLORATION OF THE ANTIBACTERIAL, ANTIOXIDANT AND ANTICANCER POTENTIAL OF THE SEED COAT EXTRACT OF MUNGBEAN (*VIGNA RADIATA* L. WILCZEK)

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### ABSTRACT

Plant derived products are not just the part of our food but also it contains many phytochemicals that possess many medicinal properties to tackle various ailments. Studies on the phytochemicals in the food sources might lead to the discovery of more safe and reliable drugs. So keeping it in mind, present study aims on exploring the potential antibacterial, antioxidant and anticancer potential of the seed coat extract of *Vigna radiata*. Seeds being very rich source of easily digestible proteins are part of vegetarian diet. Preliminary study on phytochemicals showed the significant differences in the composition of the seed coats and seeds without seed coat. Methanolic extract of seed coat showed good antibacterial activity against both Gram positive and negative bacteria with zones of inhibition ranging from 25-28.5 mm in diameter at concentration of 10 mg/ml. The extract also exhibited good antioxidant potential when tested with two different methods that are DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging activity and FRAP (Ferric Reducing Antioxidant Power Assay) method showing activity of 80% and 77% respectively at the concentration of 100 µg/ml. For anticancer activity checked by SRB assay against the human lung cancer cell line HOP-62, the extract at the concentration of 80 µg/ml showed 65% cell growth inhibition activity. The work concludes that seeds must be consumed with seed coat which has medicinal values due to rich antioxidant, antibacterial activity and anticancer potential thereby it could act as raw source of drugs for treating various ailments.

**Keywords:** Auxin, phytohormones, physiological, cell cycle, regulation, growth.

### INTRODUCTION

With the passage of time and disorganised way of using commercial antibiotic drugs, there has been the development of multidrug resistance in bacteria (Cowan, 1999). This has led to discover new agents to combat the issue of resistance and is safe to use. The solution is to untangle the medicinal properties of plants that could be low in cost and a very good source of antimicrobial agents as well as to treat other diseases. There has been increase in the consumption of plant derived foods at the rate of 5-10% per annum after the potential health benefits of the foods suggested by the clinical studies (Tham *et al.*, 1998). Moreover there is improvement in the health status of an individual with increase in uptake of the plant derived food as suggested by many health organizations worldwide (Espin *et al.*, 2007) and decrease in the risk of developing diseases like chronic disease, cardiovascular diseases, cancer, diabetes, cataract and age related functional diseases with regular consumption of fruits and vegetables (Liu, 2003). These health benefits of plants-based diet are due to the presence of secondary metabolites or phytochemical (Liu, 2004) that are not only responsible for antibacterial or antioxidant activity but also show other beneficial effects like regulating blood pressure and lipid profile, lowering C-reactive protein and inhibiting LDL oxidation (Liu and Finley, 2005). Among the phytochemical, phenols and flavonoids are found to be responsible for anticancer properties and

act on tumour cells by different mechanisms that include antiproliferative activity, antioxidant activity, regulation of different genes such as tumour suppresser gene, NF-κB gene which results in regulating COX-2 activity and PGE 2 synthesis, apoptosis induction and anti-angiogenesis (Liu *et al.*, 2005; Sun and Liu, 2006; Yoon and Liu, 2007). So the consumption of food containing such phytochemical seems to be very beneficial to health.

Mungbean (*Vigna radiata* L. Wilczek) is an important grain legume that is grown for its edible seeds and sprouts that is consumed in south east countries like India and Bangladesh as fresh salad vegetable or common food as 'dal' (Ferry, 1990). It has high nutritive values and well documented health enhancing benefits which has changed the consumer's preference (Fernandez-Orozco *et al.*, 2008). Mungbean as a food contains high amount of proteins, carbohydrates, vitamins, minerals and fibres as a part of nutrition along with significant level of bioactive phytochemicals mainly polyphenols that are thought to be responsible for many properties such as antimicrobial, antioxidant and anti-inflammatory (Kanatt *et al.*, 2011; Randhir *et al.*, 2004; Vanamala *et al.*, 2006; Anjum *et al.*, 2011). Nowadays, several food products based on mungbean are made but most of them use its seeds without the seed coat. This may result in decrease or loss in many active properties of the food. So, the present study has been made to find the properties that are exhibited by the seeds coat of the mungbean seeds.

## MATERIALS AND METHODS

### Plant materials and Preparation of extracts

Seeds of *V. radiata* were collected from local market and kept in moisture chamber up to 16 hours for softening of seeds coat. The seed coats were separated from cotyledons; shade dried both and powdered for the further study. The extraction was performed using Soxhlet apparatus and methanol as choice of solvent for extraction. The extract was prepared under the previously optimized conditions of 65°C temperature for 10 cycles of extraction used for the preparation of extracts for other legume crop (Mehta *et al.*, 2018). The extract was obtained in concentrated form and then dried in the hot air oven at 50°C temperature to powder it and stored at 4°C until further use.

### Phytochemical screening

The two extracts thus obtained were analyzed to preliminary qualitative phytochemical screening following the procedures in accordance to Mehta *et al.*, 2018. Various phytochemicals like alkaloids, flavonoids, tannins, saponins, phenols, glycosides, sugars and proteins were checked (Table 1). The degree of colour obtained was used as preliminary quantification measure.

### Antibacterial assay

The antibacterial activity of seed's coats extract of *V. radiata* was tested against pathogenic Gram positive and negative bacteria that are *Bacillus cereus*, *Bacillus subtilis*, *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa* and *Staphylococcus aureus*, using agar well diffusion method. Extract was dissolved in distilled water at the concentration of 100 mg/ml for preparing the stock. Overnight grown culture of each strain was uniformly swabbed onto the individual plates of Muller Hinton Agar (MHA) using sterile cotton swabs and then 2 wells of 6mm diameter were made. Dissolved extract (100µl) was poured in one well of each plate along with the commercial drug cefotaxime in the other well. All the plates were incubated at 37°C for 24 h. After incubation the different levels of zones of inhibition formed around the wells were measured. All the experiments were performed in triplet.

### Antioxidant activity

Antioxidant activities were checked with DPPH and FRAP methods for determination of radical scavenging activity and reducing power respectively (Elansary *et al.*, 2012; Loo *et al.*, 2007). For radical scavenging activity, 2 ml of DPPH reagent (0.1 mmol/l) was mixed with 2 ml of the sample solution of different concentration in methanol (20-100 µg/ml) in a test tube and incubated at room temperature in the dark for 30 min. The absorbance was measured at 517 nm to calculate the activity. Low

absorbance indicated higher antioxidant activity expressed as DPPH radical inhibition percentage. Reducing power was checked by mixing the 2 ml of extract of various concentrations (20-100 µg/ml) in 0.2M phosphate buffer (pH 6.6) and 2.5 ml of potassium ferricyanide solution (1%). The reacting content was kept for 20 minutes at 50°C and reaction was terminated with addition of 1ml of 10% trichloroacetic acid (TCA). The supernatant was separated from reaction mixture by centrifugation at 3000 rpm for 15 minutes. And then 0.1% ferric chloride was dissolved in it. Absorbance was measured at 700 nm against ascorbic acid as control. The reducing power of all the extracts was calculated.

### Experimental procedure or SRB assay

The anticancer activity of extract was checked by using method of Skehan *et al.*, (1990) at ACTREC, Mumbai. Human lung cancer cell line (Hop-62) was used for study. The cells at appropriate density (90 µL/well) were inoculated in 96 well titre plates and used TCA (trichloroacetic acid) to fix in-situ for representing cell population measurement during drug addition (Tz). Solubilised extracts from stock were diluted (400-fold) to get desired test concentration of 100, 200, 400 and 800 µg/ml in the medium. From these different dilutions, 10 µl of each were added to 90 µl of cell suspension to get the final concentration of 10, 20, 40 and 80 µg/ml. A known anticancer drug Adriamycin was used as positive control. End point measurement was used for result interpretation where fixed cells were stained with Sulforhodamine B solution. Wavelength of 690 nm was used as reference wavelength and absorbance was noted at 540 nm. Percent growth was calculated and expressed as the ratio of average absorbance of test well to that of control wells x 100. Percentage growth inhibition was calculated as:  $[(Ti - Tz)/(C - Tz)] \times 100$ , where Ti is test growth; Tz is growth at time zero; C is control growth. Experimental data of cell viability against extract concentration was estimated by using linear regression method.

## RESULT AND DISCUSSION

### Phytochemical Screening

The results of preliminary phytochemical tests have shown the differences in the presence of various phytochemicals in seed coat and seeds without seed coat (Table 2). Sugar component was absent from the seed coat part whereas tannins were absent in seeds without seed coat. There is also significant difference in the quantity of these phytochemicals in both extracts as observed by the calorimetric differences. Alkaloids, flavonoids and phenols are present in higher amount in seed coats as compared to the storage tissue of the seeds which contains sugars and proteins in higher amount.

### Antibacterial Activity

**Table 1.** Methodology for phytochemical screening of different compounds

Test for	Methodology	Predictive Observation	
		For Presence	For Absence
Alkaloids	1 ml extract + 1 ml Wagner's reagent	Reddish brown precipitate	No precipitate
Saponins	5 ml extract shaken vigorously	Foamy layer	No Foam
Tannins	5 ml extract + few drops of FeCl <sub>3</sub> (1%)	Green colour precipitate	No precipitate
Glycosides	2 ml extracts + 10 ml H <sub>2</sub> SO <sub>4</sub> (50%) + 2 ml Fehling's solution	Brick-red precipitate	No precipitate
Flavonoids	2 ml extract + 4 drops NaOH + H <sub>2</sub> SO <sub>4</sub>	Colourless solution	Yellow colour
Sugars	10 ml extract + 4 drops Fehling's A & B	Red colour	No red colour
Proteins	1 ml extract + 1 ml Biuret reagent	Violet colour	Blue colour
Phenols	1 ml extract + 4 drops FeCl <sub>3</sub>	Blackish colour	No colour at interface

**Table 2.** Results of the phytochemical screening tests done for different extracts

	Alkaloid	Flavonoid	Saponin	Glycoside	Sugar	Protein	Tannin	Phenol
Seeds without seed coat	+	+	++	++	+++	+++	-	+
Seed coat	+++	+++	+	+	-	+	++	++

'-' indicates absence; '+' indicates presence in low amount; '++' indicates presence in medium amount; '+++' indicates presence in high amount.

**Table 1:** Antibacterial activity of seed coat extract of *V. radiata* against different bacteria

Bacteria	Zone of Inhibition(mm)*	
	Seeds coat extract	Control (Cefotaxime)
<i>Bacillus subtilis</i>	25.48 ± 0.43	29.3 ± 0.31
<i>Klebsiella pneumoniae</i>	27.54 ± 0.36	32.2 ± 0.46
<i>Staphylococcus aureus</i>	27.21 ± 0.41	31.3 ± 0.61
<i>Escherichia coli</i>	28.51 ± 0.34	30.5 ± 0.33
<i>Pseudomonas aeruginosa</i>	26.71 ± 0.24	31.1 ± 0.41
<i>Bacillus cereus</i>	26.12 ± 0.65	29.7 ± 0.30

The data represents values are expressed as mean ± SD (n=3)

The seed coats extract showed potent antibacterial activity against all the tested pathogenic bacterial strain. The zone of inhibition ranges from 25-28 mm in diameter showing its broad-spectrum range by inhibiting both Gram positive and Gram negative bacteria (Table 1). These zones of inhibition are close to the commercially used antibiotic cefotaxime (29-32 mm) which was used as positive control in the experiment (Figure 1).

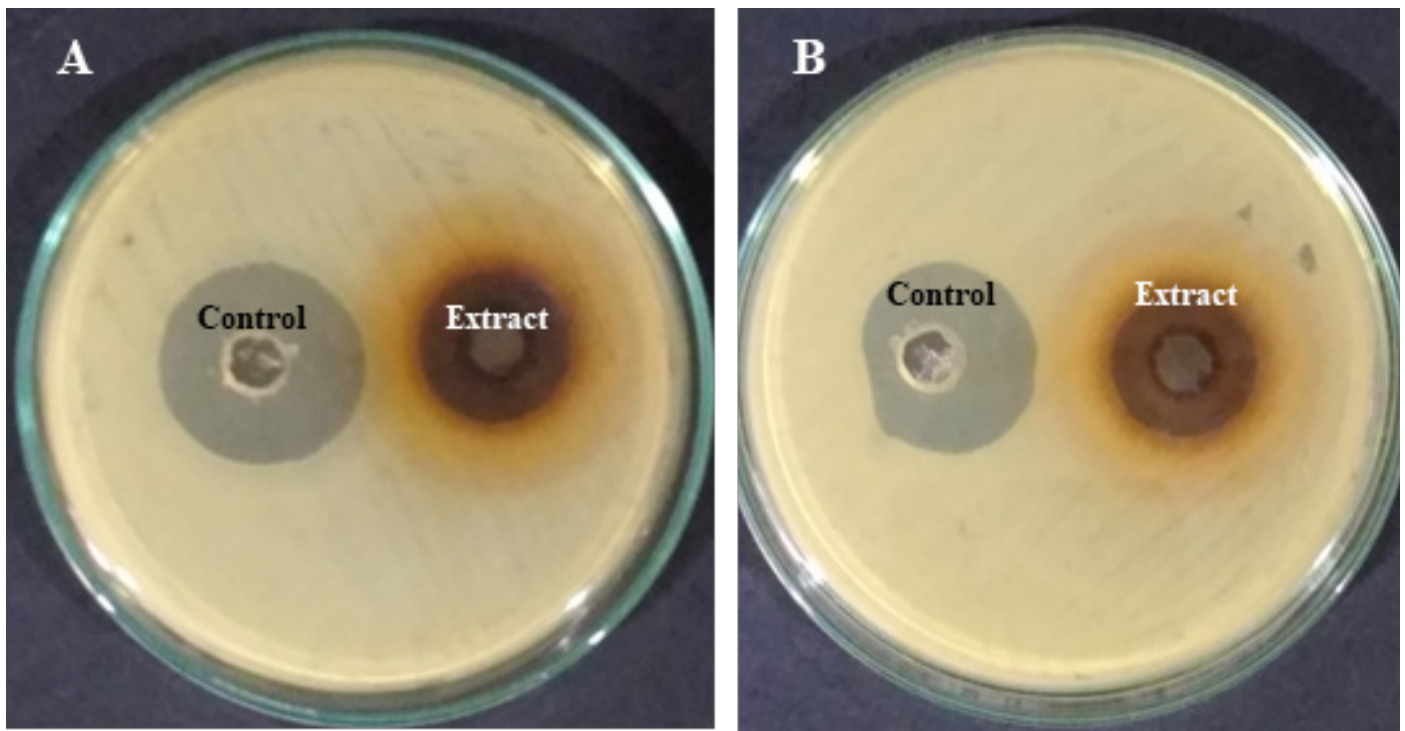
#### Antioxidant activity

The extract possesses significant antioxidant potential showed by free radical scavenging activity and reducing activity when checked with both DPPH and FRAP methods respectively. With increase in concentration of the extracts, the antioxidant activity also increased. The antioxidant potential was found almost similar to the

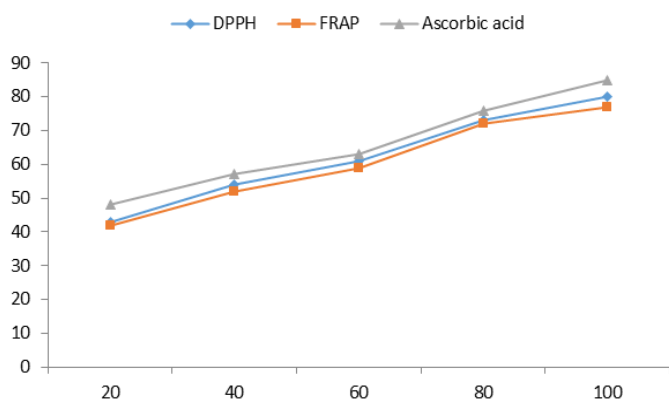
control used at equimolar concentrations. The DPPH free radical scavenging activity and reducing power of seed coat was found to be 80% and 77% respectively at concentration of 100 µg/ml whereas the value of ascorbic acid at equimolar concentration was 85% in both cases (Figure 2).

#### Anti-cancer activity

The anticancer activity of the extracts was checked against the human lung cancer cell line Hop-62. It was found that the seed coat extract exhibit anti cancer potential against the tested cell line by inhibiting the growth of the cells with average inhibition of 65% at the concentration of 80 µg/ml, whereas at lower concentrations, no or negligible inhibitory activity was found (Figure 3). The activity of the seed coat extract is very less than the control drug (Adriamycin) used which may be contributed by the fact



**Figure 1:** Zones of inhibition on MHA medium (A) against Gram positive bacteria *B. subtilis*; B) against Gram negative bacteria *E. coli*. Cefotaxime was used as positive control

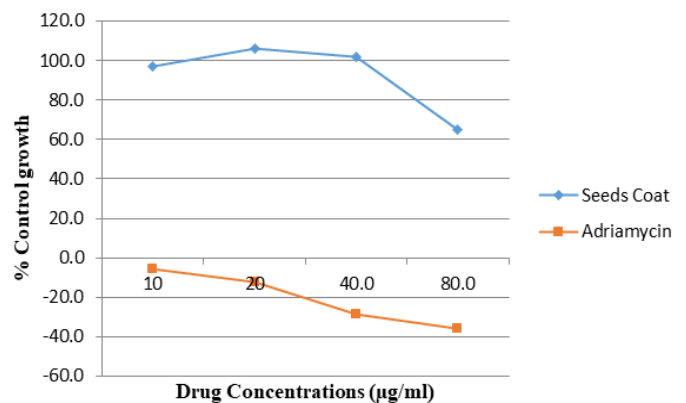


**Figure 2:** Antioxidant activity of seed coat extract of *Vigna radiata* at different concentration.

that the extract is a mixture of compounds whereas the drug is a pure compound.

## Discussion

With the changing food habits, there has been increase in the health concerns which has led to the trend towards the use of herbal products which further enhances during COVID-19 pandemic. Now there has been search for new plant metabolites from the commonly available plant products that may have diverse biological properties. So, in the present study methanolic seed coat extract of mungbean has been evaluated for the first time for antibacterial, antioxidant and anticancer potential. Nowadays many food products are using mungbean seeds without seed coat which may result in loss of many significant phytochemicals with beneficial properties. Significant differences in the phytochemicals have been observed in seed coat extracts and seeds without seed coat. Many bioactive important compounds are present in



**Figure 3:** Effect of seed coat extract of *V. radiata* on growth of Human Lung Cancer Cell Line Hop-62

higher amount in the seed coat of mungbean. The results are in accordance with the previous study where flavonoids are found in higher amount in seed coats than in the cotyledons of mungbean (Kim *et al.*, 2005). The extract has found to possess significant antibacterial activity against the tested microorganisms. The maximum zone of inhibition (28.5 mm) was found against *E. coli* whereas lowest zone of inhibition (25 mm) was found against *B. subtilis* though these are lesser than the control drug used. The activity of seed coat extract is very much significantly higher than the whole seeds extracts as reported earlier (Camalxaman *et al.*, 2013) where methanolic extracts showed zone of 10 mm against *E. coli* at concentration of 700 mg/ml. This high antibacterial activity of seed coat extract in our report may be due to the fact that many active phytochemicals might be present in the seed coat specifically.

Similarly, extract showed high antioxidant potential checked by DPPH free radical scavenging activity and

reducing power. The extract showed promising free radical scavenging activity as shown by Total Antioxidant Activity (TAA) of 80% as checked by DPPH method. Also extract showed good reducing activity by reducing Fe<sup>3+</sup> to Fe<sup>2+</sup> during FRAP assay. The activity gets increased with increase in the concentration which is similar to the results obtained in other legume crops (Mehta *et al.*, 2019, Rao *et al.*, 2019). This high antioxidant activity of the seed coat extract may be due to the presence of large amount of flavonoids (50 times) more than the cotyledons (Kim *et al.*, 2005). These flavonoids are responsible for antioxidant and anti-inflammatory properties thus used in cosmetics (Jeong *et al.*, 1998; Kim *et al.*, 1998).

Plants have been the source of many anticancer drugs which resulted in more research on finding more drugs with such potential particularly in plants or their parts consumed in routine diets. So in this context we checked the *in vitro* cytotoxicity of the extract using SRB assay against the human lung cancer line HOP-62. The extracts possess some anticancer activity as evident from the 65% cell growth inhibition at the concentration of 80 µg/ml. The results of SRB assay correlates with the changes in the cell proteins which varies with the cell number or the protein turnover of the cells tested. Upon exposing the cells to toxicants, alteration in the morphology is also the most observed effect (Ekwall *et al.*, 1990). Previously, seed coat extract has not been checked for anticancer activity although whole seeds have been tested by MTT assay for its anti-proliferative effect where it showed dose dependent effect against different cancer cell lines like CAL27, DU145, MCF-7, HL-60 and SK-OV-3 (Xu and Chang, 2012). In case of whole seed extracts, the anti-proliferative activity (IC<sub>50</sub>) was very less even at very high concentrations (1-2 mg/ml) of extracts as compared to our tested value of 80 µg/ml where 65% growth inhibition was obtained. The antioxidant components contribute mainly to the anticancer potential (Xu and Chang, 2012) which is present in very high amount in seed coat as compared to the cotyledons in mungbean (Kim *et al.*, 2005).

It can be concluded that the seed's coats are the rich source of phytochemicals that possess very good antibacterial and antioxidant activity along with some anticancer potential. So, in this context we suggest eating whole seeds of mungbean rather than polished dal, for maximum nutritional and medicinal benefits of the crop. Also the study opens future avenues for the further exploration of the compounds responsible for such activities to develop new therapeutical formulations using this important grain legume. Being a food crop, it can be exploited for cheap, safe and efficient supplement that could be used for tackling ailments and upliftment of the health of the human beings.

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