# EXPLORATION OF THE ANTIBACTERIAL, ANTIOXIDANT AND ANTICANCER POTENTIAL OF DIFFERENT PARTS OF THREE VARIETIES OF *VIGNA MUNGO* (L.) HEPPER

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***Abstract***
*Today spectra of plant based natural drugs have been increasing tremendously. Thus, present study explores the potential antibacterial, antioxidant and anticancer potential of extracts of different parts of three varieties of Vigna mungo. Different plant parts (shoot, seeds, seed coat) and varieties (PUSA I, PUSA II and T 9) affect the presence of phytochemicals qualitatively and quantitatively. Seed coats were found to possess higher content of alkaloids, flavonoids, phenols and tannins as compared to other tissues. Between the different varieties, PUSA I showed higher content of various metabolites. Among the different parts of three varieties, methanolic extracts of seed coat of PUSA-I variety showed highest antibacterial activity against Gram positive (Bacillus cereus, Bacillus subtilis) and Gram negative (Klebsiella pneumoniae, Pseudomonas aeruginosa, Escherichia coli)bacteria, with zones of inhibition ranged between 22 to 28 mm in diameter at a concentration of 10 mg/ml. Italso exhibited the best antioxidant potential when evaluated using DPPH (2,2-diphényl-1-picrylhydrazyl) radical scavenging activity and FRAP (Ferric Reducing Antioxidant Power Assay),showing activity of 74% and 76%, respectively at concentration of 100 µg/ml. Anticancer activity was checked by SRB assay against the Human cancer cell lines of prostate (PC-3), lungs (Hop-62) and renal cell adenocarcinoma (786-O).The PUSA-I seed coat extract at the concentration of 80 µg/ml showed 68%,72% and 69%viability,respectivelyin the above cell lines. Thus, it can be concluded that Vigna mungo seeds should be consumed with seed coat as it has medicinal values; acting as a source of drug or dietary supplement for controlling various ailments.*

**Key words:** Antibacterial, Anticancer, Antioxidant,Blackgram, Phytochemicals.

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## 1. INTRODUCTION

Microbial infections, oxidative stress, and cancer are of important concerns related to human health. To deal with microbial infections, presently antibiotics are playing an important role, but their effectiveness is reducing continuously towards certain bacterial strains leading to development of multiple drug-resistance. Second matter of concern to human health is oxidative stress that causes damage of certain proteins, fatty acids and DNA of our body and becomes one of the causatives for many diseases like diabetes, neurodegenerative diseases, atherosclerosis, cardiovascular diseases, cancer etc. (Ivanova et al. 2005; Katalinic et al. 2006). Among these diseases, cancer is very dangerous as it is the second leading cause of death worldwide. Today's mostly synthetic drugs used to treat such disorders have side effects (allergy, toxicity, hypersensitivity, immunosuppressant etc. (Shariff, 2001). Hence researchers are now focusing on plant based natural drugs rather than synthetic ones. Many important drugs have been developed from plants since 19$^{th}$century, including morphine (pain killer) and more recently paclitaxel (anti-cancer) and artemisinin (anti-malarial) (Katiyar et al. 2012).Different plants have nutritional value and pharmacological activities because of the presence of bioactive compounds. Hence, nowadays foods rich in nutraceuticals and dietary fibres are gaining importance because of their health benefits and in this line, pulses are an important food source for most population as they are the rich source of proteins and other nutrients along with certain phytochemical like polyphenols, flavonoids, tannins, saponins and alkaloids etc.

Blackgram is one of the important grain legumes widely used in India and other Asian countries as a potent source of protein. It is also a rich source of other nutrients like carbohydrates, fibres, minerals, and vitamins along with some bioactive compounds like polyphenols, flavonoids, tannins, alkaloids etc. that have health enhancing benefits(Lee and Shibamoto, 2000; Peng et al. 2008; Pandey 2019). Among the different phytochemical, blackgramis a prominent source of phenolic acid and flavonoids that possess properties like antimicrobial, antioxidant, anti-inflammatory etc.In general, such pharmaceutical properties are contributed by the type and content of active metabolites present in the plants, which are ultimately affected by the plant genome constitution and its differential expression even in various tissues of the same plant (Li et al. 2020).There are reports that showed differences in phytochemical content and biological properties of plants with changes in varieties of the same species and tissue sources (Suneja et al. 2011; Biswas et al. 2012; Karimi et al. 2015; Criste et al. 2020). Thus, the present study has been undertaken to examine the antibacterial, antioxidant and anticancer activities of different parts of different cultivars of *Vigna mungo*.

## 2. MATERIAL AND METHODS

### Plant materials and preparation of extracts

Vigna mungo (L.) Hepper cultivars (T-9, PUSA-I and PUSA-II) were procured from Pulses Research Laboratory, Division of Genetics, IARI (Indian Agriculture Research Institute) New Delhi. Seeds were grown in pots and different parts (shoot, seed and seed coat) were collected, cleaned, dried in shade at room temperature and powdered. Seed coats of all the varieties were harvested from one day old water-soaked seeds followed by drying in shade along with the remnant water used for soaking (not clear) and then powdered for preparing extracts. Various extracts were prepared using powdered material in Soxhlet apparatus using methanol as solvent by following the previously optimized conditions of 75°C temperature for 12 cycles of extraction used for preparing extracts of Macrotyloma uniflorum (Rao et al. 2019).The so obtained solvent extracts were evaporated to dryness and remaining powder stored at 4°C for further use. The extracts were used for phytochemical profiling and to assess antibacterial, anti-oxidative and anticancer activities assay.

### Phytochemical screening

The extracts thus obtained were subjected to preliminary phytochemical screening following the procedures described briefly in Table 1.

**Table 1. Tests used for phytochemical screening of various plant extracts*.**

| Test for | Methodology | Predictive observation | |
|---|---|---|---|
| | | For presence | For absence |
| Alkaloids | 1 ml extract + 1 ml Wagner's reagent ($HgCl_2$ + KI+ $H_2O$) | Reddish brown precipitate | No precipitate |
| Flavonoids | 2 ml extract + 4 drops NaOH + $H_2SO_4$ | Colourless solution | Yellow colour |
| Glycosides | 2 ml extracts + 10 ml $H_2SO_4$ (50%) + 2 ml Fehling's solution ($CuSO_4$+ $KNaC_4H_4O_6 \cdot 4H_2O$) | Brick-red precipitate | No precipitate |
| Phenols | 1 ml extract + 4 drops $FeCl_3$ | Blackish colour | No colour at interface |
| Proteins | 1 ml extract + 1 ml Biuret reagent (NaOH + $CuSO_4$+ $KNaC_4H_4O_6 \cdot 4H_2O$) | Violet colour | Blue colour |
| Saponins | 5 ml extract shaken vigorously | Foamy layer | No Foam |
| Sugars | 10 ml extract + 4 drops Fehling's A ($CuSO_4$)& Fehling's B ($KNaC_4H_4O_6 \cdot 4H_2O$) | Red colour | No red colour |
| Tannins | 5 ml extract + few drops of $FeCl_3$ (1%) | Green colour precipitate | No precipitate |

* Methods as described by Mehta et. al, 2018

Various phytochemical like alkaloids, flavonoids, tannins, saponins, phenols, glycosides, sugars, and proteins were checked for their presence in the extracts. The degree of colour obtained was used as a preliminary quantification measure following the method described in earlier published reports (Senguttuvan et al. 2014; Mehta et al. 2018).

**Antibacterial assay**

Extracts of different parts of all the three varieties of *V. mungo* were tested for antibacterial activity by agar well diffusion method against Gram positive (*Bacillus cereus, Bacillus subtilis*) and Gram negative (*Klebsiella pneumoniae, Pseudomonas aeruginosa, Escherichia coli)* bacteria. For preparing stock solutions, crude plant extracts were dissolved in DMSO at their natural pH at a concentration of 100 mg/ml. Different bacterial strains were grown overnight on Mueller Hintonagar (MHA) medium and then freshly cultured colonies picked with the help of sterilized loop and mixed in liquid Mueller Hinton medium (2 ml) and swabbed uniformly onto the individual plates using sterile cotton swabs followed by formation of 5 wells of 6 mm diameter on each plate. The different extracts were poured into each well in equal amount (100µl) on all plates swabbed with different bacterial strains. The commercial antibacterial drug cefotaxime (5mg/ml)and dissolving solvent (DMSO)were used at 100 µl concentration as a positive control and negative control, respectively. The plates were incubated at 37°C for 24 h in an incubator.

**Statistical analysis**

Data was obtained by measuring the zone of inhibition formed around the wells. Each experiment was performed in triplicate and then 'mean ± Standard Deviation' values were calculated using Microsoft Excel 2013.

**Antioxidant activity**

Antioxidant activities of all the extracts and ascorbic acid (control) has checked with DPPH (radical scavenging activity) (Elansary et al.2012) and FRAP (reducing power)(Loo et al. 2007) determination methods.

For radical scavenging activity, 2ml of DPPH reagent (0.1mmol/l) was mixed with 2ml 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 517nm and activity calculated using the following equation:

$TAA\ (\%) = (A_{control}\text{-}A_{sample}/A_{control}) \times 100.$

Where TAA% (Total antioxidant activity) is the percentage inhibition of the DPPH radical, $A_{control}$ is absorbance of the control sample that includes all the reagents except the extract sample and $A_{sample}$ is absorbance of the extract sample. Lower absorbance indicated higher antioxidant activity expressed as DPPH radical inhibition percentage.

Reducing power was checked by mixing the 2ml of extract at various concentrations (20-100 µg/ml) in 0.2M phosphate buffer (pH 6.6) and 2.5ml of potassium ferricyanide solution (1%). The reaction mixture was kept for 20 minutes at 50ºC and the reaction then terminated with the addition of 1ml of 10% trichloroacetic acid (TCA). The supernatant was separated from reaction mixture by centrifugation at 3000 rpm for 15 minutes and dissolved in 0.1% ferric chloride. The absorbance was measured at 700 nm and the activity was calculated using the same equation as described above.

**Anticancer activity**

The anticancer activity of extracts from different parts of best responsive variety (PUSA-I)was checked against different cell lines i.e. Human lung cancer cell line (Hop-62),Human Renal cell Adenocarcinoma cell line 786-O and Human prostate cancer cell line (PC-3) at ACTREC (Advanced Centre for Treatment, Research and Education in Cancer), Mumbai, by using the SRB assay [Skehan et al., 1990].For the experiment, different extracts at concentrations of 20, 40, 60 & 80 µg/ml was taken into 96 well microtiter plates containing cells. After 24 h, cell population was measured

at the time of addition of the drug or extract (Tz) by *in situ* fixing the cells with TCA (trichloro acetic acid). Adriamycin (Anticancer drug) was used as a positive control and the experiment repeated thrice. Staining was done with Sulforhodomine B solution and the absorbance measured on an Elisa Plate Reader at a wavelength of 540nm Wavelength at690 nm was used as reference. Percent growth was calculated and expressed as the ratio of average absorbance of test well to that of the control wells x 100. Percentage growth inhibition was calculated as: [(Ti-Tz)/(C-Tz)] x 100, where Ti is test growth; Tz is growth at time zero; C is control growth. Cell viability against drug concentration of tested samples was checked by linear regression method.

## 3. RESULTS

### Phytochemical screening

The results of preliminary phytochemical screening tests of all the three varieties and their parts (shoot, seed and seed coat) showed differences in the distribution of various phytochemical (Table 2). In all the three varieties, saponins were not found in shoots and seeds coat while tannins in seeds. Other metabolites checked had shown their presence, but with significant differences in the quantity of these phytochemical in extracts of all varieties. Among the different metabolites, tannins, glycosides, alkaloids, phenols, flavonoids, saponins and proteins were found more abundant in PUSA I variety as compared to other two varieties. Alkaloids, flavonoids and phenols are present in higher amount in seed coats as compared to the storage tissue of the seeds (seed without seed coat) which contains sugars, proteins and saponins in higher amount.

### Antibacterial Activity

After the phytochemical screening tests, different parts of all the three varieties were analysed for the antibacterial activity. A zone of inhibition between 10-28 mm diameter was observed with all tested strains, indicating broad-spectrum antibacterial activity of the extracts (Table 3). These zones of inhibition are close to the commercially used antibiotic cefotaxime (28-32 mm), a positive control in the experiment (Figure 1). Among the extracts from different parts of all the varieties studied, seed coat extracts of PUSA-I had the highest antibacterial activity against all the tested pathogenic bacterial strains with the highest mean zone of inhibition (~28 mm) against *Bacillus subtilis* and minimum zone of inhibition (~22 mm) against *Pseudomonas aeruginosa* though these zones had lesser size than the control drug cefotaxime (Table 3).

**Table 2. Results of the phytochemical screening tests done for methanolic extracts of different parts of three different varieties of *Vigna mungo*.**

| | **Blackgram cultivars** | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | **T9** | | | **PUSA-I** | | | **PUSA-II** | | |
| **Phytochemicals** | Shoot | Seeds | Seed Coat | Shoot | Seed | Seed Coat | Shoot | Seeds | Seed Coat |
| Alkaloids | ++ | + | + | ++ | ++ | ++ | ++ | + | ++ |
| Flavonoids | + | + | ++ | ++ | + | +++ | + | + | ++ |
| Glycosides | + | + | + | + | ++ | + | + | + | + |
| Phenols | ++ | + | +++ | ++ | ++ | +++ | + | + | + |
| Proteins | + | + | + | ++ | + | + | + | + | + |
| Saponins | - | ++ | - | - | ++ | - | - | ++ | - |
| Sugars | + | + | + | + | + | + | + | + | + |
| Tannins | + | - | ++ | ++ | - | ++ | + | - | ++ |

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

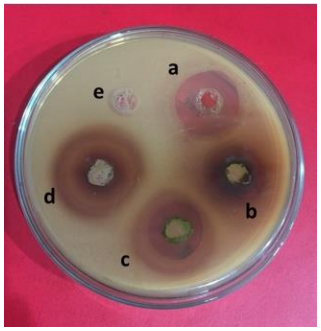


**Figure 1. Antibacterial activity of extracts prepared from different parts of the PUSA-I variety of *Vigna mungo* against *Bacillus subtilis*: a - Positive control (cefotaxime), b - Seed, c - Shoot, d – Seed coat, e – Negative control (DMSO).**

**Table 3: Antibacterial activity of different plant parts extract of three different cultivars of *Vigna mungo*[1]**

| **Mean zone of inhibition (values in mm )[2]** | | | | | | |
|---|---|---|---|---|---|---|
| **Variety** | **Part** | ***E. coli*** | ***K. pneumoniae*** | ***P. aeruginosa*** | ***B. subtilis*** | ***B. cereus*** |
| T 9 | Shoot | 12.4±0.21 | 14.3±0.37 | 11.6±0.32 | 17.4±0.34 | 15.8±0.21 |
| | Seed | 15.3±0.22 | 12.5±0.39 | 10.2±0.33 | 14.1±0.43 | 12.5±0.24 |
| | Seed coat | 23.4±0.54 | 23.3±0.54 | 18.6±0.42 | 26.2±0.24 | 24.7±0.30 |
| PUSA I | Shoot | 16.6±0.32 | 17.4±0.23 | 15.4±0.34 | 19.2±0.21 | 17.4±0.33 |
| | Seed | 14.5±0.26 | 15.3±0.22 | 13.3±0.26 | 17.3±0.14 | 14.2±0.26 |
| | Seed coat | 24.4±0.54 | 26.4±0.36 | 22.4±0.42 | 28.1±0.24 | 26.4±0.34 |
| PUSA II | Shoot | 14.4±0.23 | 15.4±0.44 | 13.8±0.23 | 18.4±0.21 | 17.4±0.23 |
| | Seed | 13.2±0.16 | 13.8±0.32 | 12.3±0.24 | 16.3±0.22 | 13.7±0.26 |
| | Seed coat | 23.4±0.24 | 24.6±0.36 | 20.4±0.44 | 26.4±0.54 | 25.2±0.28 |
| Standard (cefotaxime) | | 30.2±0.52 | 32.8±0.24 | 31.6±0.46 | 32.4±0.32 | 28.4±0.24 |

[1] Extracts extracted with 80% methanol at 75˚C for 12 Soxhlet cycles and at their natural pH.
[2]The values of experiments expressed as mean ± SD (n=3)

**Antioxidant activity**

The extracts possess significant antioxidant potential as shown by free radical scavenging activity and reducing activity when checked with both DPPH and FRAP methods, respectively. With the increase in concentration of the extracts, the antioxidant activity also increased. Among the three varieties, PUSA-I had found to possess highest antioxidant activity. Out of different parts of PUSA-I variety, we found highest activity in seed coat extracts followed by shoot and seeds in both the methods used for analysis. Though seeds coat showed a high antioxidant potential, it was less than the pure ascorbic acid which was taken as standard. The total average antioxidant activity (TAA) percent value of the seeds coat, seeds and shoots was found to be 76, 66, and 68% respectively, and that of ascorbic acid (standard) was 81.25% at the concentration of 100 µg/ml (Figure 2).

**Anti-cancer activity**

The anticancer activity of extracts obtained from different parts of the most responsive PUSA-I variety was checked against the different human cancer cell lines i.e., human prostate cancer cell line (PC-3), human lung cancer cell line (Hop-62) and human renal cell adenocarcinoma cancer cell line (786-O).The seed coat extract was found to exhibit anticancer potential by inhibiting the growth of the cells with average viability of 68%, 72% and 69%, respectively. The extract from other tested parts showed no or negligible activity

against all the tested cell lines. The activity of seed coat extract was observed only at the concentration (80 µg/ml) against all the cell lines, although at the same concentration the control drug (Adriamycin) showed significant cytotoxicity (Figure 3).

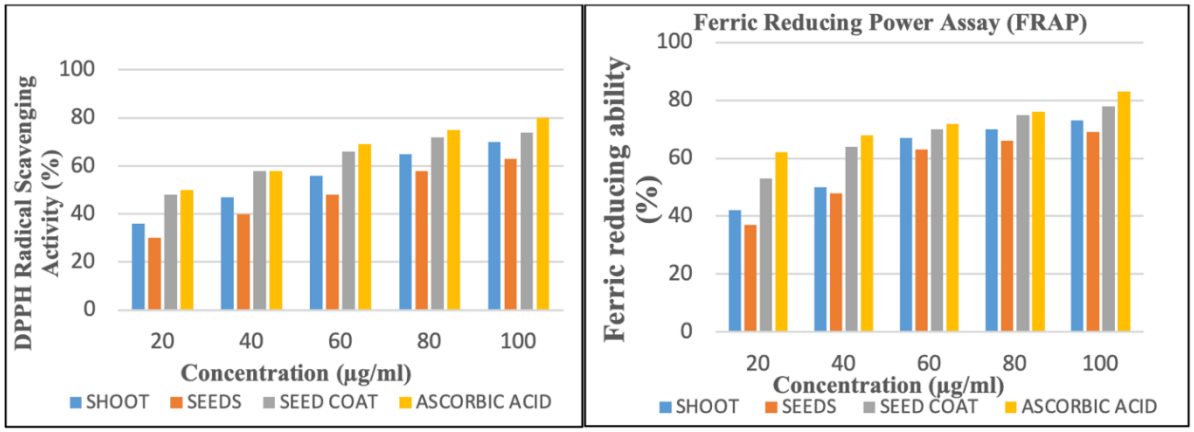


**Figure 2. Antioxidant activity of different plant parts extract of PUSA I cultivar of *Vigna mungo* at different concentrations using DPPH (a) and FRAP assay (b).**

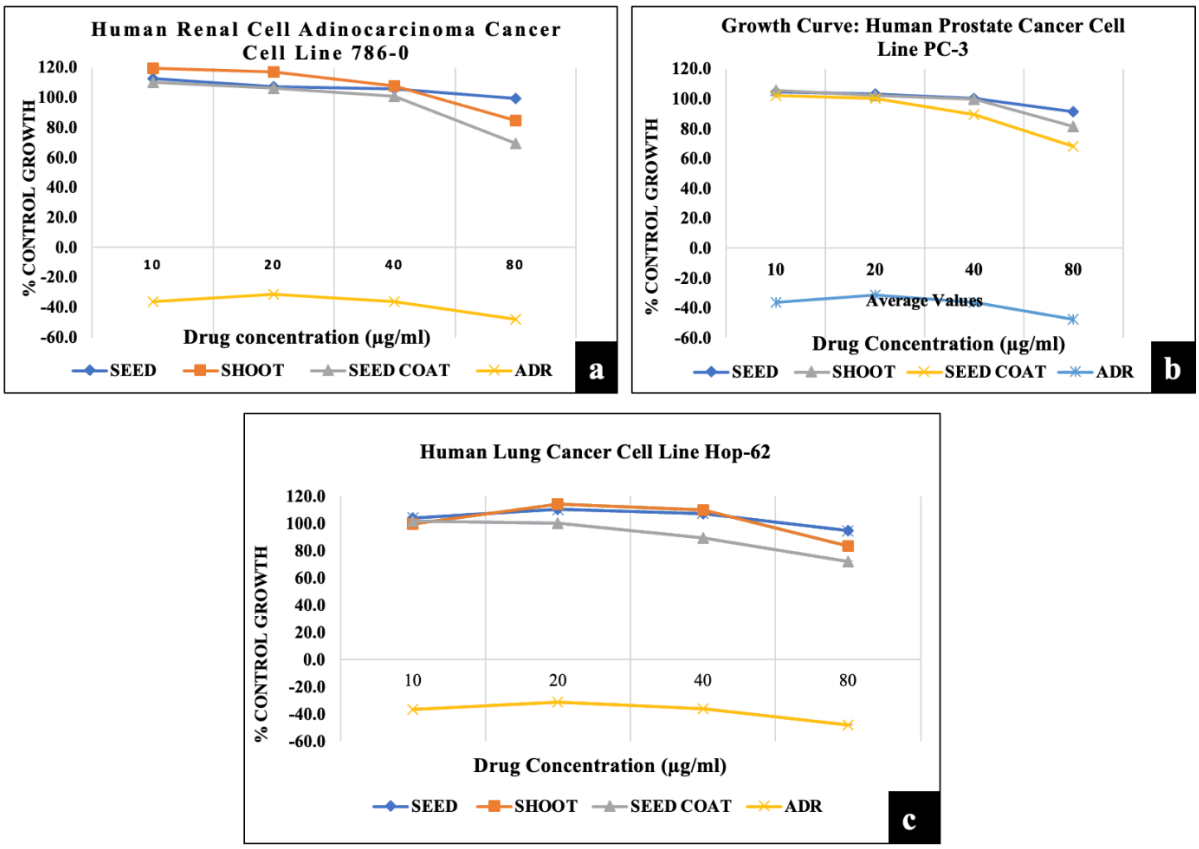


**Figure 3. Effect of different extracts including shoot, seed and seed coat of PUSA I variety of *Vigna mungo* on growth of different human cancer cell lines checked by using SRB assay. (a): Human Prostate Cancer Cell line PC-3; (b): Human Renal cell Adenocarcinoma Cancer Cell line 786-O; (c): Human Lung Cancer cell line HOP-62.**

## 4. DISCUSSION

From the results it was observed that seed coat contains more phytochemical qualitatively and quantitatively (no quantitation was done here) in all the three varieties followed by shoot and seed. The results are in accordance to the previous study, where phytochemicals are more prominent in the seed coat (Girish et al. 2012).Among different phytochemicals present in the blackgram, saponins were absent in seed coat and shoot of all the three varieties. Our results are in accordance to Gayathri et al.(2016), who also observed alkaloids, tannins, phenolic acid and some other bioactive compounds but no saponins in the seed coat of various pulses including blackgram.

The extracts obtained from different parts of the three varieties of the *Vigna mungo* were screened for antibacterial and antioxidant activity while the anticancer activity was tested with extracts of the more responsive PUSA-1 variety. The differential antibacterial potential was shown by the three varieties of the blackgram. Among three varieties used, PUSA-I was found best compared to T9 and PUSA-II. Such varietal differences affecting antibacterial activity has been reported earlier also in different plant species like water chestnut (Biswas et al. 2012), *Labisia pumila*(Karimi et al. 2015) *Rosaindica* (Sahoo et al. 2011), and also in legumes like *Macrotyloma uniflorum* (Rao et al. 2019) etc. In blackgram, some reports are available that showed the varietal difference in terms of proximate composition, nutritional and anti-nutritional factors that ultimately affects the biological activity of different blackgram cultivars (Suneja et al. 2011; Zia-Ul-Haqet al. 2014; Modgil et al. 2019).The reason behind such variations may be the differences in qualitative or quantitative or both of different metabolites in different varieties of same plant species (Siddhuraju et al. 2008; Suneja et al. 2011; Zia-Ul-Haq et al. 2014).Plant part used is also an important factor affecting antibacterial activity as evidenced in several other legume species like *Macrotyloma uniflorum* (Rao et al. 2019), *Cicer arietinum* (Kan et al. 2010), *Sesbania aculeate* (Mehta et al. 2019).In our study of different parts of *Vigna mungo*, the seed coat extracts of PUSA-I variety showed highest antibacterial activity against all the bacterial strains compared to the other parts tested. Difference in antibacterial activity of different part extracts has also been reported earlier in *Vigna mungo*. According to Nasrin et al. (2015), leaf and stem extracts show an average zone of inhibition ranged from 10 to 20 mm which is comparable to the range of 11-19 mm when similar part was used in this study. The zone of inhibition was however, significantly lesser compared to seed coat (18-28 mm); the differences may be because of the different plant parts. In another reports, Chikaneet al.(2010) and Kingsley et al.(2014) studied blackgram antibacterial activity in seeds coat and seeds, respectively with highest zone of inhibition 17 mm against *Pseudomonas aeruginosa* in case of seed coat and 13mm against *Klebsiella* in seeds that is significantly lesser than our findings i.e. 22mm and 15mm respectively using the same bacteria and plant part. These differences might be because of varietal differences used in different studies.

Extracts also showed antioxidant activities when checked with DPPH and FRAP radical scavenging assay. Out of different parts, we found highest activity in seed coat extracts followed by shoot and seeds via both the methods used for analysis. The activity increases with an increase in the concentration which in similar to the results obtained in other legume crops (Tiwari et al. 2013; Mehta et al. 2019; Rao et al. 2019).The present results are in accordance to earlier reports on the higher antioxidant potential of the seed coats because of the presence of more phenolic and flavonoids (Girish et al. 2012;Arockianathan et al. 2019).

Today, in the line of treatment of cancer many drugs have derived from plants having phytochemical exhibiting anticancer potential such as vinblastine and vincristine from *Catharanthus roseus* and taxol from *Taxus brevifolia* etc. This has leads to the screening of

more and more therapeutically important plants particularly which are consumed in routine diets. In this context, *in vitro* cytotoxicity of *V. mungo* extracts from different parts as checked by the SRB assay against three different human cancer cell lines showed only seed coat extract to have some anticancer potential differentially against different cell lines. The reason for this variable response with the same extract might be because of different cell lines tested. The observed activity of the seed coat extract was much less than the control drug (Adriamycin), which may be because the extract is a mixture of compounds whereas the drug is a pure compound. Earlier, cytotoxicity of leaves and shoot extract of this plant was also checked against brine shrimp nauplii and shown mortality with $LC_{50}$ values of 4.52µg/ml and 3.25µg/ml for leaves and stem extract, respectively (Nasrin et al. 2015). However, seed coat extracts of some other legumes have shown some anticancer potential as tested by the SRB assay (Rao et al. 2019; Mehta et al. 2019; Mehta et al. 2021).

The difference in above studied biological activities of different varieties and tissue source could be because of allelic differences in different varieties and differential expression of genes in different varieties or parts of the plant, which has also evidenced from phytochemical screening.

## 5. CONCLUSION

The study has concluded that type and quantity of phytochemicals and pharmaceutical properties of *Vigna mungo* deviates with the use of various plant part and variety. The extracts of different parts (shoot, seed and seed coat) of T-9, PUSA-I and PUSA-II three different varieties of *V. mungo,* all have shown antimicrobial, antioxidant and anticancer potential but differentially because of differential content of active metabolites in different plant part and variety. Among different varieties, best response for the activities tested was obtained with the PUSA-I variety. While among different parts, seed coat extract showed the best results for antimicrobial, antioxidant and anticancer potential, which could be because of presence of the highest content of flavonoids, phenols, tannins and alkaloids compared to other parts tested. Seed coat extracts of PUSA-I variety had showed antibacterial activity against all the tested bacterial strains and the highest mean zone of inhibition (~28 mm in diameter) at a concentration of 10 mg/ml against Gram positive *Bacillus subtilis*. The extract also exhibits good level of antioxidant potential with TAA value of 76% that was very close to 81.25% of ascorbic acid (standard) at the concentration of 100 µg/ml. Seed coat extract also possess the anticancer potential, it showed 68%, 72% and 69% viability of the human cancer cell lines of prostate, lungs and renal cell adenocarcinoma, respectively at the concentration of 80 µg/ml. Thus, on a basis of our study, we recommend eating whole seed of blackgram rather than polished ‘dal’ as seed coat is a rich source of pharmaceutically important phytochemicals. Because of presence of active metabolites, blackgram has a potential for developing natural drugs or dietary supplements that ultimately help in tackling ailments and uplift of human health. The findings also open future avenues to conduct pharmacological studies to understand the possible underlying mechanisms and exploration of active compounds responsible for these activities.

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## 6. REFERENCES

[1]. Arockianathan, P. M. Rajalakshmi, K. and Nagappan, P. 2019. Proximate composition, phytochemicals, minerals and antioxidant activities of *Vigna mungo* L. seed coat. Bioinformation 15(8): 579-585.

[2]. Biswas, K. K. Faruk, M. O. Amin, M. Z. and Shaha, R. K. 2012. Antibacterial activity of two varieties of water chestnuts (*Trapa* sp.). Journal of Biosciences 20:115-123.
[3]. Chikane, M.R. Parwate, D.V. and Ingle, V.N. 2010. *In Vitro* Study of Antibacterial Activity of γ-irradiated and Unirradiated Leguminous Seed Coats. International Journal of Pharmaceutical and Biological Archieve 1(4): 335-337.
[4]. Criste, A. Urcan, A.C. Bunea, A. Priponfurtuna, F.R. Olah, N.K. Madden, R.H. and Corcionivoschi N. 2020. Phytochemical composition and biological activity of berries and leaves from four Romanian sea buckthorn (*Hippophaerhamnoides* L.) varieties. Molecule 25(5): 1170-1176.
[5]. Elansary, H. O. Salem, M. Z. M. Ashmawy, N. A. and Yacout, M. 2012. Chemical composition, antimicrobial and antioxidant activities of *Lantana camara*, Cupressus *sempervirens* and *Syzygiumcumini* leaves oils from Egypt. Journal of Agricultural Science 4(10): 144-152.
[6]. Gayathri, A. Deepa, M.S. and Durai, F.M. 2016. Phytochemical screening and antibacterial activity of selected pulses husk extracts. Indo- Asian Journal of Multidisciplinary. Research 2(6): 896-900.
[7]. Girish, T.K. Pratape, V.M. and Rao, U.P. 2012. Nutrient distribution, phenolic acid composition, antioxidant and alpha-glucosidase inhibitory potentials of black gram (*Vigna mungo* L.) and its milled by-products. International Food Research46(1): 370-377.
[8]. Ivanova, D. Gerova, D. Chervenkov, T. and Yankova, T. 2005. Polyphenols and antioxidant capacity of Bulgarian medicinal plants. Journal of Ethnopharmacology 96(1): 145-150.
[9]. Kan, A. Özçelik, B. Kartal M. Özdemir, Z.A. and Özgen, S. 2010. *In vitro* antimicrobial activities of *Cicer arietinum* L. (Chickpea). Tropical Journal of Pharmaceutical Research9(5): 475-481.
[10]. Karimi, E. Jaafar, H.Z. Ghasemzadeh, A. and Ebrahimi, M. 2015. Fatty acid composition, antioxidant and antibacterial properties of the microwave aqueous extract of three varieties of *Labisia pumila* Benth. Biological Research48(9): 1-6.
[11]. Katalini, V. Milos, M. Kulisic, T. and Jukic, M. 2006. Screening of 70 medicinal plant extracts for antioxidant capacity and phenols. Food Chemistry 94(4): 550-55.
[12]. Katiyar, C. Gupta, A. Kanjilal, S. and Katiyar, S. 2012. Drug discovery from plant sources: An integrated approach. Ayu 33(1):10-19.
[13]. Kingsley, D. Ravikumar, G. Chauhan, R. and Abraham, J. 2014. Extraction and screening of bioactive metabolites from *Vigna mungo* against various pathogens. International Journal of Pharmaceutical Sciences and Research5(2): 428-431.
[14]. Lee, K. and Shibamoto, T. 2000. Antioxidant properties of aroma compounds isolatedfrom soybeans and mung beans. Journal of Agriculture and Food Chemistry 48(9): 4290–4293.
[15]. Li, Y. Kong, D. Fu, Y. Sussman, M. R. and Wu, H. 2020. The effect of developmental and environmental factors on secondary metabolites in medicinal plants. Plant Physiology and Biochemistry 48: 80-89.
[16]. Loo, A.Y. Jain, K. and Darah, I. 2007. Antioxidant and radical scavenging activities of the pyroligneous acid from a mangrove plant, *Rhizophora apiculata*. Food Chemistry 104(1): 300–307.
[17]. Mehta, N. Rao, P. and Saini, R. 2018. Efficient extraction of thermostable metabolites of Sesbania aculeata and in vitro evaluation for their antibacterial potential. AnaleleUniversitatii din Oradea, FasciculaBiologie 25(1): 33-41.
[18]. Mehta, N. Rao, P. and Saini, R. 2019. Evaluation of antioxidant and anticancer potential of *Sesbania aculeata* – a multipurpose legume crop. *Annals*.Food Science and Technology 20(1): 109-115.
[19]. Mehta, N. Rao, P. and Saini, R. 2021. Exploration of the antibacterial, antioxidant and anticancer potential of the seed coat extract of mungbean (*Vigna radiata* L. Wilczek). Plant Archieves21(1): 1628-1633.
[20]. Modgil, R. Kaundal, S. and Sandal, A. 2019. Bio-chemical and functional characteristics of black gram (*Vigna mungo*) cultivars grown in Himachal Pradesh, India. International Journal of Current Microbiology and Applied Sciences 8(4): 2126-2137.
[21]. Nasrin, F. Paul, S. Zaman, S. and Koly, S. 2015. Study of antimicrobial and cytotoxic activities of *Vigna mungo* Linn. Hepper (Family-Leguminosae). Pharma Tutor 3(4): 40-46.
[22]. Pandey, S. 2019. Review on medicinal importance of *Vigna* genus. Plant Science Today 6(4): 450-456.
[23]. Peng, X. Zheng, Z. Cheng, K. Shan, F. Ren, G. Chen, F. and Wang, M. 2008. Inhibitory effects of mung bean extract and its constituents vitexin and isovitexin on the formation of advanced glycation end-products. Food Chemistry 106(2): 475–481.
[24]. Rao, P. Mehta, N. and Saini, R. 2019. Exploration of medicinal properties of Macrotyloma uniflorum – an important grain legume crop plant. AnaleleUniversitatii din Oradea, FasciculaBiologie 26(1): 27-33.
[25]. Sahoo, A.M. Chakraborti, C.K. Nayak, S. and Kayal, S. 2011. Correlation between phytochemical screening and in vitro antibacterial activity study of *Rosa indicalinn*. leaves. International Journal of Ayurveda and Pharma Research2(5): 1595-1597.

[26]. Senguttuvan, J. Paulsamy, S. and Karthika, K. 2014. Phytochemical analysis and evaluation of leaf and root parts of the medicinal herb, *Hypochaerisradicata* L. for *in vitro* antioxidant activities. Asian Pacific Journal of Tropical Biomedicines 4(3): 59-67.

[27]. Shariff, Z. U. 2001. Modern herbal therapy for common ailments. *pp*. 9-84. Nature Pharmacy Series (Volume 1) Spectrum Books Limited, Ibadan, Nigeria in association with Safari Books (Export) Limited, United Kingdom.

[28]. Siddhuraju, P. Maheshu, V. Loganayaki, N. and Manian, S. 2008. Antioxidant activity and free radical scavenging capacity of dietary phenolic extracts from processed indigenous legumes, Macrotyloma uniflorum (Lam.) Verdc. and Dolichos lablab L. Food 2: 159-167.

[29]. Skehan, P. Storeng, R. Scudiero, D. Monks, A. McMahon, J. Vistica, D. Warren, J. T. Bokesch, H. Kenney, S. and Boyd, M. R. 1990. New Colorimetric Cytotoxicity Assay for Anticancer-Drug Screening. Journal of the National Cancer Institute 82(13): 1107-1112.

[30]. Suneja, Y. Kaur, S. Gupta, A.K. and Kaur, N. 2011. Levels of nutritional constituents and antinutritional factors in black gram (*Vigna mungo* L. Hepper). Food Research International 44(2): 621–628.

[31]. Tiwari, A.K. Manasa, K. Kumar, D.A. and Zehra, A. 2013. Raw horsegram seeds possess more *in vitro*antihyperglycaemic activities and antioxidant properties than their sprouts. Nutrafoods 12(2): 47-54.

[32]. Zia-Ul-Haq, M. Ahmad, S. Bukhari, S. A. Amarowicz, R. Ercisli, S. and Jaafar, H. Z. 2014. Compositional studies and biological activities of some mash bean (*Vigna mungo* (L.) Hepper) cultivars commonly consumed in Pakistan. Biological Research 47(1):1-14.