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# EXTRACTION AND SCREENING OF BIOACTIVE METABOLITES FROM *VIGNA MUNGO* AGAINST VARIOUS PATHOGENS

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**ABSTRACT:** In the present study, *Vigna mungo* (blackgram) have been used to investigate bioactive compounds through soxhlet extraction. The obtained methanol extract was evaluated for antimicrobial activity by Kirby-Bauer method against bacterial strains (*Enterococci* sp., *Pseudomonas aeruginosa, Klebsiella* sp., *Bacillus* sp., *Staphylococcus aueres*) and fungal strains (*Aspergillus niger, Candida albicans)* on Muller Hinton agar medium and potato dextrose agar medium respectively. The blackgram was analyzed for the presence of proteins through SDS-PAGE and compared with the BSA (66KDa) and Lysine (18KDa) protein markers. The sensitivity of the obtained methanol extract against pathogenic microorganisms was studied with the combined effect of commercially available antibiotics which resulted in good and enhanced antimicrobial effect. The methanol extract of blackgram was also analyzed for bioactive compounds through gas-chromatography mass spectrometry (GC-MS). The GC-MS spectrum revealed bioactive compound with molecular weight of 297.3268 (Heptadecanoic acid, 9-methyl, methyl ester). The active constituents of *Vigna mungo* was identified through mass spectroscopy analysis act as potential compound for effective antimicrobial and pharmaceutical studies.

**INTRODUCTION:** *Vigna mungo* commonly known as blackgram is the highly cultivated crop in Indian subcontinent. It belongs to the legume family which is a rich source of antioxidants in treating various ailments like liver diseases, rheumatism, diabetes, heart diseases and infections in the central nervous system [1]. Pulses are regarded as abundant source of bioactive substances and most of the bioactive substances have been classified as 'antinutritional factors' [2]. The root nodules of blackgram possess medicinal properties with narcotic, diuretic effect and it is also used as remedy for aching bones, dropsy and cephalgia [3,4,5].

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It has been reported that pulses acquire anti-inflammatory and anti-cancer properties whereas recent studies have also demonstrated that protein antinutritional compounds such as lectins, protease inhibitors and the non-antinutritional component, angiotensin I-converting enzyme (ACE) inhibitor may have beneficial properties.

It has been also proposed that pulses contain a wide variety of non-nutritive bioactive components such as enzyme inhibitors, phytic acid, lectins, phytosterols, phenolic compounds and saponins [6]. Grain legumes have been regarded as a good source of lectins in human food and lectins from pulses like blackgram are known to inhibit the growth of experimental animals and reduce the digestibility and biological value of dietary proteins [7]. On the whole pulses make a major dietary component to human health as a source of protein, vitamins, minerals, dietary fiber and folic acid [8].

Other than these micronutrients it possess biological active compounds like enzyme inhibitors, lectins, phytates, oligosaccharides, and phenolic compounds in abundant amount which contributes to pharmaceutical growth.

Considering all the major macronutrients of blackgram from Vellore, the present study was conducted to screen for biological active compounds through extraction and analytical instrumentation.

## MATERIALS AND METHODS:

**Sample preparation:** Blackgram (*Vigna mungo*) were purchased from local market of Vellore, Tamil Nadu, India. For proper germination of pulses, it was soaked in water for 5-6h and was then packed in wet cloth for overnight incubation. The germinated pulses were air-dried completely and were finely ground for further analysis.

**Preparation of pulses extracts:** 50g of finely ground pulses powder was extracted using methanol in a Soxhlet extractor not exceeding the boiling point of the solvent. The methanol extract of pulses was obtained after Soxhlet extraction of 72 h. The extracts were filtered through Whatmann filter paper no. 1 and were further concentrated under reduced atmospheric pressure. The obtained dry extract was maintained in 5% dimethyl sulphoxide (DMSO) and was stored at 4°C for further experimental analysis.

**SDS-PAGE analysis:** The proteins present in blackgram were determined through sodium dodecyl sulphate- polyacrylamide gel electrophoresis (SDS-PAGE). Finely ground gram powder of 20mg was mixed with 200µl of sample buffer containing 150mM Tris, 1% w/v SDS, 30% v/v glycerol, 15% v/v β-mercaptoethanol and 0.002% bromophenol blue, pH 6.8 and incubated at room temperature for overnight.

After the incubation was complete, the blackgram sample was heated upto 100°C in water bath for 5 minutes followed by centrifugation at 10,000rpm for 15 minutes. The obtained supernatant was run on SDS-PAGE using 12% separating gel and 5% stacking gel[9]. The molecular weight of the protein was determined by comparing with bovine serum albumin (BSA) and lysine marker.

**Antimicrobial activity:** The obtained extract was analyzed for antibacterial and antifungal activity against various pathogens. The sensitivity of pathogens to methanol extract of blackgram was tested by measuring the zone of inhibition obtained against the pathogenic microorganisms. The antibacterial effect was manifested against *Enterococci* sp., *Pseudomonas aeruginosa*, *Klebsilla pneumonia*, *Bacillus* sp. by well diffusion method. The pathogens were swabbed onto Muller-Hinton agar plates and 100µl of methanol extract of blackgram was added into the well punctured onto the agar plate.

The plates were then incubated at 37°C and zone of inhibition was observed after 24h of incubation. For antifungal activity, *Aspergillus niger* and *Candida albicans* was used to check antimicrobial effect. The spore suspension of both the fungal culture was prepared using tween 20 and was added onto potato dextrose agar plates. 100µl of the methanol extract sample was added into the well punctured onto the PDA plates. The zone of inhibition was measured after 5d of incubation at 28°C.

**Synergistic effect:** The combined effect of commercially available antibiotics (chloram-phenicol, tetracycline, eythromycin, cinorflaxacin, fluconazole and voriconazole) and obtained methanol extracts of blackgram against pathogenic microorganism implies synergistic effect. The disk diffusion method was employed to determine the combined effect. Muller-Hinton agar and potato dextrose agar was used for bacterial and fungal pathogens respectively. The antibiotic disc without extract was placed onto the agar plate which served as control and antibiotic disc impregnated with 100µl of the methanol extract was also placed onto the same petridish. The zone of inhibition was measured after 24h for bacterial cultures and 5d for fungal cultures[10].

**GC-MS analysis:** The obtained methanol extract was analyzed for gas-chromatography mass spectrometry to identify the number of compounds and molecular weight of the compounds. GC-MS analysis was carried out on a GC Clarus 500 Perkin Elmer system comprising of auto sampler and gas chromatograph interfaced to a mass spectrometer.

**RESULTS AND DISCUSSION:** The blackgram when allowed to germinate produces sprouts which contain a large quantity of proteins and among them some are biologically active for e.g., lectins, ribosome inactivating proteins, enzyme inhibitors, arcelins, chitinases and canatoxin [11]. They are majorly known as enzyme inhibitors comprise of both amylase and proteinase inhibitors [12]. These plants belong to three main families namely *Leguminosae*, *Solanaceae* and *Gramineae* [13] and Blackgram (*Vigna mungo*) belongs to the genus *Vigna* and subgenus *Ceratotropis* which is extensively cultivated in all over India. The extracts of plant leaves are commercially used as anti-inflammatory, analgesic and ulcerogenic drug [14] but the extracts of germinated blackgram has not been very well studied.

The present work employs the extraction of phytochemical compounds of sprouted blackgram through various solvents and the biological activities of obtained extracts. The methanol extract obtained after Soxhlet extraction was dried completely and stored at 4°C for further biological activities. The methanol extract of blackgram inhibited all the pathogenic microorganisms. The maximum zone of inhibition was manifested against *Klebsilla* sp. (13mm) followed by *Bacillus* sp. (11mm), *Enterococci* sp. (5mm) and *Pseudomonas aeruginosa* (4mm). The antifungal effect was recorded as *Aspergillus niger* (12mm) and *Candida albicans* (13mm). The SDS-PAGE of blackgram revealed proteins of similar molecular weight as BSA and lysine marker which are 66KDa and 18KDa respectively as shown in **Figure 1 and Figure 2** respectively.

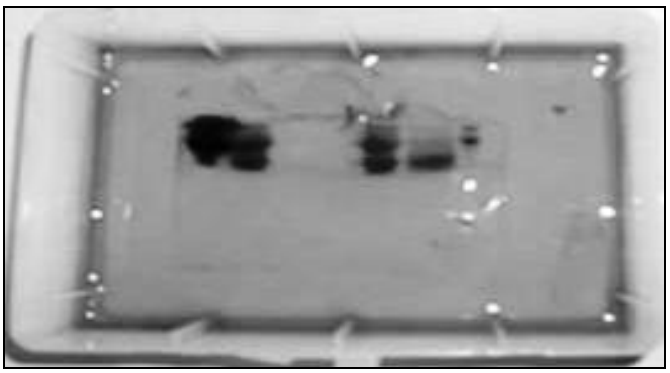

**FIGURE 1: SDS-PAGE OF METHANOL EXTRACT OF BLACKGRAM WITH BSA PROTEIN MARKER**

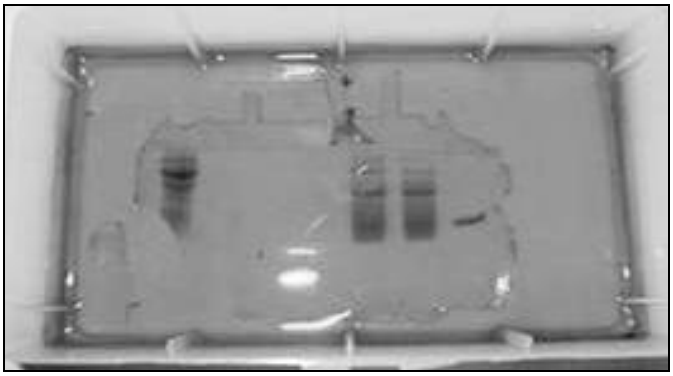

**FIGURE 2: SDS-PAGE OF METHANOL EXTRACT OF BLACKGRAM WITH LYSINE PROTEIN MARKER**

The synergistic effect was observed to be highest against *Bacillus* sp. with choloramphenicol (13mm) and the weak synergistic effect was manifested against *Pseudomonas aeruginosa* with choloramphenicol (10mm). The GC-MS analysis revealed biological active compound through fragmentation study i.e. heptadecanoic acid 9-methyl, methyl ester with molecular weight of 297.326 as shown in **Figure 3**.

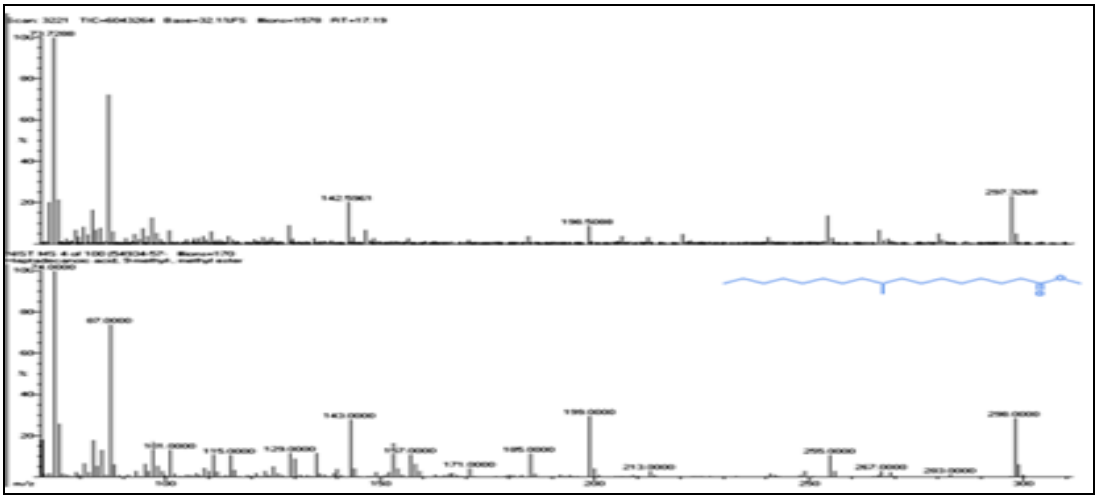

**FIGURE 3: GAS CHROMATOGRAM-MASS SPECTROMETRY ANALYSIS OF METHANOL EXTRACT OBTAINED AFTER SOXHLET EXTRACTION**

The proteinaceous and biological active components of blackgram extracted by methanol resulted in effective antimicrobial activity and methyl ester identified through sophisticated analytical techniques is also responsible for inhibition activity against pathogenic microorganisms.

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**Declaration of interest:** The authors report no conflicts of interest.

## REFERENCES

1. Battu G, Male A, Priya TH, Malleswari VN and Reeshma SK: A phytopharmacological review on *Vigna* species. Pharmanest 2011; 2:62-67.
2. Rechcig M: Handbook of Naturally Occurring FoodToxicants. CRC series in nutrition and food. Boca Raton, FL: CRC Press, 1983.
3. Pullaiah T: Encyclopedia of world medicinal plants, Daya Books, 2000, pp. 1045-1046.
4. Chothani DL and Vaghasiya HU: A phyto-pharmacological overview on *Phylsalis minima* Linn. Indian Journal of Natural Products and Resources. 2012; 3:477-482.
5. Krishnan V, Gopi M, Amerjothy S: Morphological diversity and some newly recorded plant galls in Tamil Nadu, India. Indian Journal of Science and Technology. 2011; 9.
6. Champ MM: Non-Nutrient bioactive substances of pulses, Brazilian Journal of Nutrition, 2002; 3: 307-319.
7. Carai AM, Fantin Noemi, Loi B, Colombo G, Riva A, Morazzoni P: Potential efficacy of preparations derived from *Phaseolus vulgaris* in the control of appetite, energy intake, and carbohydrate metabolism. 2009; 145-153.
8. Singh J, Basu PS: Non-nutritive bioactive compounds in pulses and their impact on human health: an overview. Food and Nutrition Sciences, 2012; 3: 1664-1672.
9. Sambrook J, Fritsh EF and Maniatis T: Molecular cloning, A laboratory manual, cold spring Harbour, NY; 1989.
10. Fayaz AM, Balaji K, Girilal M, Yadav R, Kalaichelvan PT and Venketesan R: Biogenic synthesis of silver nanoparticles and their synergistic effect with antibiotics: a study against gram-positive and gram-negative bacteria. Nanomedicine: Nanotechnology, Biology and Medicine, 2010; 6: 103–109.
11. Barbosa A, Albuquerque E, Silva M, Souza D, Neto O, Valencia A, Rocha T, Grossi MF: α-Amylase inhibitor-1 gene from *Phaseolus vulgaris* expressed in *Coffea arabica* plants inhibits α-amylases from the coffee berry borer pest. BMC Biotechnology 2010; 10:44.
12. Clemente A, Sonnante G, Domoney C: Bowman-birk inhibitors from legumes and human gastrointestinal health: current status and perspectives. Current Protein and Peptide Science, 2011; 12: 358-373.
13. Prasad ER, Gupta D and Padmasreea K: Purification and characterization of a bowman-birk proteinase inhibitor from the seeds of blackgram (*Vigna mungo*). Phytochemistry; 2010; 71: 363–372.
14. Srivastava A and Joshi LD, Effect of feeding black gram (*Phaseolus Mungo*) on serum lipids of normal and diabetic guinea pigs. Indian Journal of Medical Research. 1990; 92: 383-386.

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