SHORT COMMUNICATION

Phytochemical analysis by GC-MS, LC-MS complementary approaches and antimicrobial activity investigation of *Vigna unguiculata* (L.) Walp. leaves

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**ABSTRACT**

Consumption of legumes has long been linked to their nutritional and medicinal benefits. *Vigna unguiculata* (L.) Walp. (Cowpea) is a legume plant in the Fabaceae family and is a rich source of nutrients also is known for its beneficial effects for diseases treatment. In terms of phytochemicals analysis and bioactivities evaluations the major research has focused on the Cowpea seeds, whereas leaves and pods are remained understudied. Herein we have highlighted leaves methanolic extract phytochemicals identification, antimicrobial, and antioxidant activity assessment. Cowpea leaves methanolic extract Liquid Chromatography-Mass Spectrometry (LC-MS) analysis first time revealed the presence of α-hederin, which is a putative novel SARS-COV-2 inhibitor and Zearalenone mycotoxin. Leaves methanolic extract exhibited strong activity against *Streptococcus pyogens* and *Candida albicans*. The Cowpea leaves extract is a potent DPPH inhibitor with an IC50 of 62.04 ± 0.08 μg/mL. The bioactive compounds identification in this work supports the plant’s nutritional and medicinal uses.

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1. Introduction

Natural products are valuable for their health-promoting properties and can serve as a blueprint for the identification of novel drug leads (Chandrasekara and Shahidi 2018). Medicinally important plants were supportive to keep humans healthy and cure diseases; in Ayurveda, the diet has a crucial role in healthy living (Che and Zhang 2019). Phytochemicals have a vital role in drug development, the plant crude extracts bioactivities screening, compounds isolation and bioactive phytochemicals semi-synthesis are important, which may lead to active pharmaceutical ingredients (Patil et al. 2018a, 2018b; Dinore and Farooqui 2020). *Vigna unguiculata* (L.) Walp. belongs to the family *Fabaceae* is commonly known as Cowpea have origin in Africa (Owade et al. 2020) and spread across Asia, North America, and South America. The plant is characterized by twining stems that can grow erect, or climbing and can reach a length of 80 cm to 2 m for climbing cultivars. The leaves are trifoliate, and alternate measuring 6-15 cm long and 4-11 cm broad. Flowers in axillary racemes on a stalk with a diameter of 2-3 cm. Pods are pendulous, smooth, and occur in pairs. They are cylindrical, 2-6 cm long, 3-12 mm wide and contain 10-15 seeds. It is primarily grown for its seeds, which are widely consumed in Africa and Asia e.g., India and China. Seeds are the most economically valuable part of Cowpea due to their high protein contents (25%), carbohydrate (64%), an excellent source of minerals, vitamins, low lipid content, and are a rich source of soluble and insoluble dietary fibers. The Cowpea plant has a cardio-protective function and is used as an antioxidant, antimicrobial, anthelmintic agent (Maisale et al. 2012). Green leaves and immature pods are also used as food however the mainstream of scientific publications research is focused only on seed analysis. Cowpea leaves are consumed to treat Vitamin-C deficiency as it has proteins, minerals like Fe, Ca, P and Zn, and vitamins such as folate, thiamin, riboflavin, and Vitamin-C (Jan and Abbas 2018). Likewise, Cowpea leaves possess high nutritional value due to a high frequency of fatty acids like palmitoleic, linoleic, α-linolenic acids, lauric, and myristic acid (Malik et al. 2016). Cowpea leaves are found rich in protein, amino acids, and minerals content than seeds; in this context encouraging the consumption of leaves with grains would be nutritionally beneficial (Mamiro et al. 2011). The leaves have been seen to show strong beneficial activities i.e., anti-atherogenic, anti-cancer, and anti-oxidant activities, attributed to phenolic acids, flavonoids, flavonols, anthocyanins, and bioactive peptides found in them (Awika and Duodu 2017; Shetty 2013). The nutritional value and therapeutic potential of these vegetative parts are moreover entirely ignored or only moderately explored. The Gas Chromatography-Mass Spectrometry (GC-MS) and Liquid Chromatography-Mass Spectrometry (LC-MS) based Cowpea leaves phytochemical analysis has never been examined before. This is the primary motivation to start this study. Furthermore, we have demonstrated the anti-microbial potential of the methanolic extracts of the Cowpea leaves, and this study aimed to quantify total phenolic content (TPC) using the Folin-Ciocalteu reagent and measure antioxidant potency by the DPPH assay.
2. Result and discussion

2.1. Phytochemical screening of Vigna unguiculata leaves methanolic extract (VULME)

The key phytoconstituents found in VULME chemical screening are flavonoids, polyphenols, alkaloids, terpenoids, fatty acids, carbohydrates, and saponins (Table S1). Analysis found that flavonoids, terpenoids, fatty acids, and alkaloids as a major component’s, carbohydrates, xanthonoids and carotenoids are present in minor quantity, whereas saponins are present in trace. Furthermore, to confirmed the VULME components GC-MS and LC-MS analysis techniques were used as they are more sensitive identification techniques. An investigation of plant natural products which are responsible for their therapeutic potential is critical task. Very few reports were available on Cowpea leaves phytochemical composition analysis using GC-MS and LC-MS complementary strategy hence this analysis in this context will be value addition.

2.2. GC-MS analysis of Vigna unguiculata leaves methanolic extract (VULME)

The phytochemical analysis by using GC-MS is a more sensitive identification technique due to its rapid screening rate and excellent mass accuracy. Twenty probable compounds belonging to numerous chemical classes were detected in the GC-MS chromatogram and mass spectra analysis (Figure S1 and Table S2). Fatty acids and esters are the major constituents that exist in extract, whereas terpenoids and essential oils are minor constituents. The fatty acids esters present are palmitic acid ester, stearic acid ester, linoleic acid ester, and linoleic acid silyl esters. 3′,8,8′-trimethoxy-3-piperidyl-2,2′-binaphthalene-1,1′,4,4′-tetrone is the abundant phytoconstituent present in the leaves (23.24%), which was reported to exhibit several bioactivities such as anticancer, anti-arthritic, and anti-inflammatory. Other components identified were 1-heptatriacotanol (9.35%, alcoholic compound), hexadecanoic acid 14-methyl-methyl ester (8.29%, fatty acid ester), 2,7-diphenyl-1,6-dioxopyridazino[4,5:2′,3′]pyrrolo[4′5′d] pyridazine (4.84%, alkaloid) and cholestan-3-one, cyclic 1,2-ethanediylactal (4.80%, steroid). Steroids are another class found in the second major constituent and representative members were androst-4-en-11-ol-3,17-dione, 9-thiocyanato, cholestan-3-ol 2-methylene- (3α 5α), cholestancholestan-3-one, cyclic 1,2-ethanediyl aetal and pregn-5-en-20-one. Several terpenoids were also detected as tabulated in Table S2. Bioactive alkaloids like 2,7-Diphenyl-1,6-dioxopyridazino[4,5: 2′,3′]pyrrolo[4′,5′d] pyridazine is present in minor quantity and it exhibited antibacterial, antimalarial, analgesic, and anticancer activity (Mary and Giri 2016). Along with these, some miscellaneous compounds were identified in minor proportions. We have found n-alkanes such as hentriacontane, pentacosane, and pentatriacontane, which were purified and characterized earlier from Cowpea leaves (Sarkar et al. 2014). Earlier studies of GC-MS analysis of ethanolic leaves extract reported the presence of oct-2-ene, non-4-ene, nona-3,5-diene, octadecanoic acid, and 1-ethyl-3-methylcyclopentane (Ogbo 2016), which did not exist in even traces in our sample analysis.
2.3. LC-Ms analysis of Vigna unguiculata leaves methanolic extract (VULME)

The LC-MS system was praised for its high sensitivity, selectivity, and ability to perform multistage MS to obtain molecular mass and relative structural details, as well as the compound’s concentration concerning peak area (Pang et al. 2016). The VULME LC-chromatogram shown in Figure S2 (a) and S2 (b) acquired in negative and positive mode of ionization respectively. VULME LC-MS chromatogram analysis revealed presence of twenty nine compounds included flavonoids, alkaloids, terpenoids, essential oils, saponins, and polysaccharides (Table S3). Cowpea polyphenolic extract has been shown to have anti-inflammatory properties inducing miR-126 (Ojwang et al. 2015). The previous research groups identified major polyphenol and flavonoid content of Cowpea seed as quercetin derivative, catechin, epicatechin, kaempferol, and p-hydroxybenzoic acid (Avanza et al. 2021) while dominant phenolic acid includes aldaric acid, protocatechuc acid, caffeic acid, and syringic acid. An earlier study revealed that the cowpea seed coat is an excellent source of flavonoids; the most abundant components were catechin and its derivatives like catechin glucoside, epicatechin, and delphinidin (Tsamo et al. 2020). In VULME LC-MS analysis six alkaloids are identified representative members are tabulated in Table S3. In this endeavour sapindoside (A) or α-hederin is detected. Currently, the whole world is under the pressure of the COVID-19 pandemic, which has become a global concern. Recently, molecular docking research suggested that sapindoside (A) is a potential novel SARS-COV-2 inhibitor isolated from *Nigella sativa* (Salim and Noureddine 2020). Mycotoxins zearalenone mainly infesting cereals such as barley, maize, wheat, and rice but it is also detected in VULME LC-MS chromatogram, which is the first report on the existence of zearlenone in pulses.

2.4. Antimicrobial screening methanolic extracts of cowpea leaves

2.4.1. Anti-bacterial activity

In the antibacterial activity screening against various bacterial strains MIC value expressed in μg/mL. VULME demonstrated strong antibacterial activity against screened bacterial species with MIC 62.5 μg/mL to 100 μg/mL. VULME showed comparable antibacterial activity to that of used standard drug ampicillin and chloramphenicol and ciprofloxacin (Table S4). *S. pyogenes* was the most susceptible bacterium with MIC 50 μg/mL VULME, while *P. aeruginosa* was found to be the most resistant up to 100 μg/mL. Gram-positive bacteria were more susceptible to the extracts than the gram-negative bacteria (Table S4) these findings are in agreement with those reported in prior research (Kritzinger et al. 2005). Gram-negative bacteria have an outer membrane layer with lipopolysaccharides that protect them from antimicrobial compounds (Madikizela et al. 2013; Zeinab et al. 2020), but Gram-positive bacteria lack this protective layer, making them more sensitive to antibiotics (Gupta and Datta 2019). Table S4 and Figure S3 showed the antibacterial activity of VULME and standard drugs.

2.4.2. Anti-fungal activity

The VULME was found most effective against *C. albicans* (MFC 200 μg/mL) which was found to be equipotent as a standard drugs nystatin and more effective than
griseofulvin (MFC 500 μg/mL) (Table S5). *C. albicans* is an opportunistic pathogen that lives on the skin and is an important part of the natural microbial flora in the oral cavity, gastrointestinal tract, and vagina hence use of this plant to prevent or treat fungal disease is important (Badiee et al. 2016). But VULME was not exhibited antifungal activity against *A. niger* and *A. clavatans* up to 1000 μg/mL. The antifungal activity of VULME and standard drugs is illustrated in Figure S4.

### 2.4.3. Anti-oxidant activity and total phenolic content analysis

The total phenolic content for the leaves extract was found to be 268.28 ± 0.05 mg GAE/g DW. The higher IC₅₀ value for leaves is credited to its rich polyphenolic and flavonoids contents, which show remarkable scavenging activity against free radicals. Natural phenolic compounds possess an aromatic ring with at least one hydroxyl group, which functions as an electron donor and contributes directly to antioxidant activity (Tungmunnithum et al. 2018). Antioxidants prevent free radicals from invading biological cells by stabilizing them. There is growing evidence that reactive oxygen species (ROS) produced in the human body can cause diseases like atherosclerosis, coronary heart disease, aging, and cancer (Li et al. 2008). Figure S5 showed the dose-response curve of Cowpea leaves DPPH radical scavenging activity in % inhibition. IC₅₀ value and antioxidant capacity are inversely proportional to each other. The plant extract showed a concentration-dependent increase in radical scavenging activity. VULME had a significantly lower IC₅₀ value 62.04 ± 0.08 μg/mL, which was closed to 58.66 ± 0.08 μg/mL for standard ascorbic acid.

### 3. Experimental

This part is described in the Supplementary Material.

### 4. Conclusion

GC-MS and LC-MS analysis speculate that the Cowpea leaves are a rich source of bioactive compounds. VULME LC-MS analysis disclosed α-hederin (Sapindoside-A) and Zearalenone presence first time in this species. The VULME has potent antimicrobial property against *Streptococcus pyogens* and *Candida albicans*. We examined the anti-oxidant study of cowpea leaves with significant phenolic and flavonoid contents, and it revealed that they could constitute a substantial source of natural antioxidants. The purification and characterization of bioactive compounds from VULME is in progress. There is a future scope for in-depth study of this plant for bioactive compounds and their pharmacological activities evaluations.

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