



GC-MS analysis unveil bioactive compounds in salicylic acid-enhanced cowpea (*Vigna unguiculata* (L.) walp)

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ABSTRACT

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Cowpea (*Vigna unguiculata*) is an economically significant native African food legume crop that offers cheap protein and vital nutrients and is also known to contain some bioactive compounds of therapeutic value. Meanwhile, salicylic acid is a plant bioregulator that plays a prominent role in promoting growth, yield, and nutraceutical value in plants. The present study aims to investigate some bioactive compounds in cowpea that are pre-treated with salicylic acid. Cowpea seeds (Ife-BPC) that were treated/untreated with salicylic acid were ground into powder and extracted with methanol; thereafter, bioactive constituents were determined using GC-MS. The GC-MS identified 43 bioactive compounds from the extract of cowpea treated with salicylic acid, while 27 bioactive compounds were obtained from the extract of cowpea without salicylic acid treatment. This study showed that salicylic acid could be used to optimize the bioactive compounds of *Vigna unguiculata* and hence increase its therapeutic potential and health benefits.

Contribution/Originality: This study contributes to the field of crop biofortification and improvement using biotechnological techniques. It originates from using GC-MS to discover bioactive compounds in salicylic acid-enhanced cowpea seeds. The primary contribution is the discovery of 43 bioactive compounds with significant nutraceutical value and therapeutic potential, thereby supporting food security.

1. INTRODUCTION

Cowpea is an important nutritional food in the human diet and is consumed as a complete protein source in many areas of the world [1, 2]. They are a significant crop because, apart from being a great source of cheap protein, they also contain additional nutrients (such as carbohydrates, low fats, amino acids, vitamins, and minerals). Cowpea grows well in low arid soil conditions. This makes it a significant crop in dry, semi-arid areas where it is impossible to cultivate other crops [3-5]. In addition, it can serve as an effective sole crop because of its nitrogen-fixing ability and can also be intercropped with cereal crops as well as tubers. It serves as an essential source of food for humans in arid regions and can also serve as feed for livestock. Cowpea has gained more attention and focus from different fields in recent times [3, 6, 7]. Though there is no proof for early cowpea cultivation, the main cultivation is from West Africa, suggesting that it is the place of early domestication. Cowpea grows at 30°C and 400- to 700-mm rainfall; therefore, it can be referred to as a seasonal crop in the continent and most of the world [8, 9]. It grows best in regions with an annual rainfall between 400 and 700 mm. It grows better on sandy soils because of its ability to tolerate infertile and acidic soil compared to other crops. The seeds can be harvested about

100 days after planting. Nowadays, cowpeas are produced all over the world, but the northern savannahs of sub-Saharan Africa produce the bulk of them, with Nigeria and Niger being the top producers. Approximately two-thirds of the world's annual cowpea crop is produced in the sub-Saharan Africa cowpea belt, with an estimated 10 million hectares under cultivation worldwide. Approximately 3.7 million tons of grain are produced annually. Brazil is the second-largest producing region after Africa, where the crop thrives due to the northeastern region's poor soils and comparatively low rainfall. Additionally, cowpeas are grown in southern Africa's marginal regions, particularly in Sudan, Somalia, Mozambique, Botswana, and southern Zimbabwe. Although cowpeas are typically interplanted with grains, not much of the produce is sold in local markets. Nigeria is the world's largest producer and consumer of cowpeas, making it the most significant export market for the crop in West Africa. Cowpeas are produced in large quantities in the Mediterranean, South Asia, and the southern United States. However, cowpea is also regarded as a climate-resilient grain legume that acts as a significant source of health-promoting bioactive compounds, which are naturally occurring molecules found in foods, animals, and plants [10, 11]. Although these compounds can also be found in other food sources like fruits, vegetables, nuts, and oils, including polyphenols (resveratrol), flavonoids (quercetin), carotenoids (lycopene), alkaloids (caffeine), and organosulfur compounds (allicin), cowpeas serve as a cheap and excellent source due to additional nutrient benefits. Elsherif et al. [12]. These compounds are generally not considered essential nutrients (like vitamins) but play a significant role in reducing the risk of chronic diseases such as cancer, cardiovascular disease, and neurodegenerative conditions [13-15]. These compounds are primarily concentrated in the seed coat and are linked to various therapeutic properties, including anti-inflammatory, anti-diabetic effects, and effectiveness as free radical scavengers [15, 16]. Research shows that pod extracts contain high concentrations of flavonoids that exhibit neuroprotective potential by inhibiting acetylcholine esterase (AChE). Also, tannins tend to bind proteins; cowpea's unique phenolic composition often permits excellent nutrient absorption while offering antioxidant advantages. Cowpeas have been defined as a strategic legume species for food security and health-promoting values [17, 18]. The key bioactive compounds in cowpeas are polyphenols, which include phenolic acids (such as protocatechuic, ferulic, and p-coumaric acids), flavonoids (flavonols and flavan-3-ols), anthocyanins (delphinidin-3-O-glucoside), bioactive peptides (globulin and albumins), and other compounds [19-22]. Since these compounds are mainly concentrated in the seed coat of cowpeas, their content varies greatly depending on seed color; pigmented, colored types often have higher concentrations of antioxidant phenolic compounds than white varieties, promoting cardiovascular health. Cowpea health benefits, such as hypoglycemic, hypolipidemic, anti-inflammatory, and anti-cancer potentials, result from the presence of bioactive compounds like phytochemicals, proteins, and peptides, which may also be linked to mechanisms suggested for protection against some metabolic and chronic disorders. In fact, it has been reported that flavonoid profiles of cowpeas have a major impact on their bioactive properties. Cowpea polyphenols and specific peptides may provide synergistic bioactive effects [23, 24]. Nowadays, people tend to consume foods not only for nutritional benefits but also for therapeutic and health-promoting advantages, which pose no risk when taken, unlike some conventional drugs that are often associated with adverse effects [16, 25, 26]. Additionally, *V. unguiculata* is termed as one of the widely valued crops that provide affordable food, nutrition, and health security in most developing nations of the world [27-29]. Cowpea is a cost-effective, nutritious crop that serves as a vital source of protein and phytochemicals, particularly for vulnerable populations, while also contributing to sustainable agricultural systems through nitrogen fixation Rouquette et al. [23] and Zia-Ul-Haq et al. [24]. Haseeba et al. [27] have reported the therapeutic and nutritious potential of cowpea in line with its phytochemistry. Awika and Duodu [3] have also reported the bioactive polyphenols and peptides in cowpea (*Vigna unguiculata*) and their health-promoting effects. Although different research has been performed on cowpea to possess anti-inflammatory, anti-cancer potentials, and other health benefits in some metabolic disorders like diabetes, obesity, hyperlipidemia, or any other chronic diseases, some studies support these claims while others do not. However, robust research on the connection between diseased conditions and cowpea consumption is required to confirm these findings.

Nevertheless, Olaiya et al. [30] reported that bioregulators have been instrumental in understanding morphological and growth phenomena in vitro and are potential tools for elucidating biochemical pathways in plants. Kumar [14] emphasized that, since abiotic stresses are major factors limiting crop productivity and sustainability worldwide, it would be pertinent to apply bioregulators to crops to induce long-term resistance in plants, as these can modulate plant responses to stress at the cellular, tissue, or organ level. Consequently, they can enhance yield, quality, and nutritional aspects of food and forage legumes in the changing climatic scenario. Moreover, bioregulator application on crops is non-toxic, suitable, and a functional approach for enhancing productivity and quality of legumes and other crops. However, bioregulators such as salicylic acid have been shown to regulate some physiological and biochemical processes in plants [25, 31, 32]. They play a crucial role in plant defense against various stresses, thus promoting growth and development, stimulating antioxidant activities, and supporting the overall well-being of plants Vessey [33]; Avanza et al. [34]; Xiong et al. [35] and Min et al. [36]. Gorni et al. [29] reported that exogenous salicylic acid and ferulic acid improve growth, phenolic, and carotenoid content in tomato. Abbas and Akladios [37] reported that treating seeds with sodium chloride, accompanied by carrot root extracts, increased growth parameters, total chlorophyll, carotenoids, and total carbohydrate in cowpea seedlings compared to seeds treated with sodium chloride alone, resulting in changes in gene products, including protein and isozyme profiles. Ailenokhuoria and Orimadegun [38] reported the effects of indole acetic acid and kinetin on the physiological and biochemical properties of two cultivars of *Cajanus cajan*. Ailenokhuoria and Orimadegun [38] had also reported that using 50% ethanol to extract cowpea (*Vigna unguiculata* L.) pods) showed higher total phenolic content (TPC) and Antioxidant capacity

. Phytochemical profiling of the extract showed that the cowpea extracts obtained were enriched in caftaric acid, *p*-coumaric acid, caffeic acid, and morin. Libra et al. [4] reported that white and black cowpea flours contained bioactive compounds such as total polyphenols and tannins, higher potassium, phosphorus, and calcium contents, while sodium, copper, manganese, zinc, and iron were present in trace amounts. Bioregulators exert their effects on plants by increasing their ability to avoid stress (morphological and biochemical barriers preventing or delaying stressor activity inside a cell, adaptation of a life cycle to seasons), stress tolerance (alternative pathways allowing a cell to function under stressful conditions, prevention of stress-generated changes, tolerance of change, or mechanisms of fast damage repair), Posmyk and Szafrńska [39]. Arifuddin and Al Banna [1] reported that GC-MS analysis revealed a diverse range of bioactive compounds, including fatty acids (oleic, palmitic, and pentadecanoic acids), alcohols, esters, nitrogen-containing molecules, vitamins, terpenoids, and phenolics, many of which are reported to possess antioxidant, antimicrobial, anti-inflammatory, anticancer, and cardioprotective properties. Therefore, there is a need to demonstrate how these health-promoting attributes could be improved in cowpea to explore its health benefits for diagnosing some human diseases and possibly serve as an alternative to the side effects of some conventional drugs, especially when the risks of taking drugs surpass their benefits. Thus, the aim of the present study is to investigate the effects of salicylic acids on the bioactive compounds of *V. unguiculata*.

2. MATERIALS AND METHODS

2.1. Plant Materials

Cowpea seeds (Ife-BPC) pre-treated with or without 1.0 μ M salicylic acid were used for the study.

2.2. Procedures for Methanolic Extraction of Cowpea

An extract was obtained from cowpea seeds according to the cold extraction methods of Sombié et al. [31] with slight modification.

Cowpea samples were dried and soaked in 1 litre of methanol at room temperature.



Decanted

Filtrate heated on a water bath at 60 °C.



Semi-liquid extract was obtained and transferred into a bottle.

2.3. Procedure for GC-MS Analysis

GC-MS analysis was performed on the extracts according to the methods of Ojwang et al. [40] with slight modifications.

Component was transferred into a vial bottle.



The vial bottle was placed into an autoinjector sample compartment.



The automatic injector was positioned to inject the sample from the lines into the column, where separation takes place into different components at different retention times.

3. RESULTS

The results in Tables 1 and 2 showed the bioactive compounds identified from treated/untreated *cowpea seeds* with salicylic acid using Gas Chromatography-Mass Spectrometry.

Table 1. GC-MS analysis of bioactive compounds present in the methanol extract of cowpea treated with salicylic acid.

S/N		Quality	Retention time
1	3-Phenyl-5-(pyridin-4-ylmethylidene)- 2-thioxoimidazolidin-4-one	98	3.602
2	Purine-2,6-dione,	98	3.602
3	8-(3-ethoxypropylamino)	98	3.602
4	1,3-dimethyl-3,9-dihydro	98	3.602
5	4-Cyano-5-ethyl-2-methoxy-6-phenyl pyridine-3-carboxamide	98	3.602
6	7-Chloro-4-methoxy-3-methylquinoline	96	4.426
7	2H-1,3-benzoxazine-6-carboxylic acid	93	4.426
8	3,4-dihydro-3-methyl-, methyl ester	93	4.426
9	1-octanol,2-methyl-oxalic acid	98	5.262
10	4-Methoxy-6-methyl-5-nitroisobenzofuran-1,3-dione	98	4.426
11	Diethylmalonic acid, monochloride	92	5.262
12	3,7-dimethyloctyl ester	92	5.262
13	2-octyl-methylester	92	5.262
14	Cyclopropanetetradecanoic acid	92	14.270
15	6-ethyloct-3-yl hepty-1 ester	92	14.270
16	Hexadecanoic acid, methyl ester	96	14.262
17	5,9,13-Pentadecatrien-2-one, 6,10, 14-trimethyl-, (E,E)-	95	16.465
18	Oxirane, 2,2-dimethyl-3-(3,7,12,16 ,20-pentamethyl-	95	16.465
19	3,7,11,15,19-heneicosapentaenyl)-, (all-E)-	95	16.465
20	10-Octadecenoic acid	99	15.756
21	trans-13-Octadecenoic acid, methyl	99	15.756

S/N		Quality	Retention time
22	cis-13-Octadecenoic acid, methyl ester	99	15.756
23	trans-13-Octadecenoic acid, methyl ester	98	15.819
24	11-Octadecenoic acid-methyl ester	94	15.819
25	10-Octadecenoic acid-methyl ester	98	15.819
26	Methyl stearate	98	15.996
27	Heptadecanoic acid	98	15.996
28	16-methyl-, methyl ester	95	16.088
29	Methyl 3,5-tetradecadiynoate	95	16.088
30	Methyl 5,7-hexadecadiynoate	97	16.088
31	2,5-Octadecadiynoic acid, methyl ester	97	16.088
32	Octadecanal, 2-bromo-	90	16.242
33	Z, Z-4,16-Octadecadien-1-ol acetate	95	16.242
34	4H-Pyran-4-one, 2,6-dimethyl-	95	16.242
35	Pentadecanoic acid	94	14.720
36	Octadecanoic acid	94	14.720
37	Cyclopropanetetradecanoic acid,	91	14.720
38	Cis-13-octadecanoic acid	91	15.756
39	S, S, S, S)-1,1'-Bicyclopentyl-2,2' dicarboxyaldehyde	95	16.683
40	Z,Z-4,16-octadecadien-1-ol acetate	95	16.242
41	Chloroacetic acid	97	16.683
42	1-cyclopentylethylester	97	16.683
43	Cyclohexene, 3-nonyl-	97	16.683

Table 2. GC-MS analysis of bioactive compounds present in methanol extract of cowpea without salicylic acid treatment.

S/N		Quality	Retention time
1	Hexadecanoic acid, methyl ester	98	13.524
2	n-Hexadecanoic acid	99	14.159
3	Methyl 10-trans,12-cis-octadecadienoate	97	15.350
4	Methyl-9-cis,11-trans-octadecadienoate	95	15.350
5	9,12-octadecadienoic acid (Z, Z)-, methyl-ester	95	15.350
6	10-Octadecenoic acid, methyl ester	99	15.350
7	cis-13-Octadecenoic acid, methyl ester	99	15.350
8	13-Octadecenoic acid, methyl ester	99	15.350
9	trans-13-Octadecenoic acid, methyl-ester	70	15.693
10	9,12-Octadecadien-1-ol, (Z, Z)-	64	15.693
11	9-Octadecenal, (Z)- cis-	78	15.693
12	13-Octadecenoic acid, methyl ester	97	15.693
13	11-Octadecenoic acid, methyl ester	75	15.693
14	9,12-Octadecadienoyl chloride, (Z, Z)-	80	15.990
15	2-Methyl-Z,Z-3,13- octadecadienol	70	15.990
16	9,12-Octadecadienal	74	15.990
17	9,17-Octadecadienal, (Z)-	97	16.254
18	9-Oxabicyclononane	86	16.254
19	cis-13-Tetradecenal	86	16.254
20	2-Methyl-Z,Z-3,13-octadecadienol	95	16.402
21	Linoelaidic acid	84	16.402
22	Octadecanoic acid	91	16.402
23	1-octadecanesulphonylchloride	98	16.402
24	Carbonic acid	94	16.402
25	eicosyl vinylester	98	16.402
26	Octadecane,1-chloro	98	16.402
27	Tridecanoic acid	98	14.497

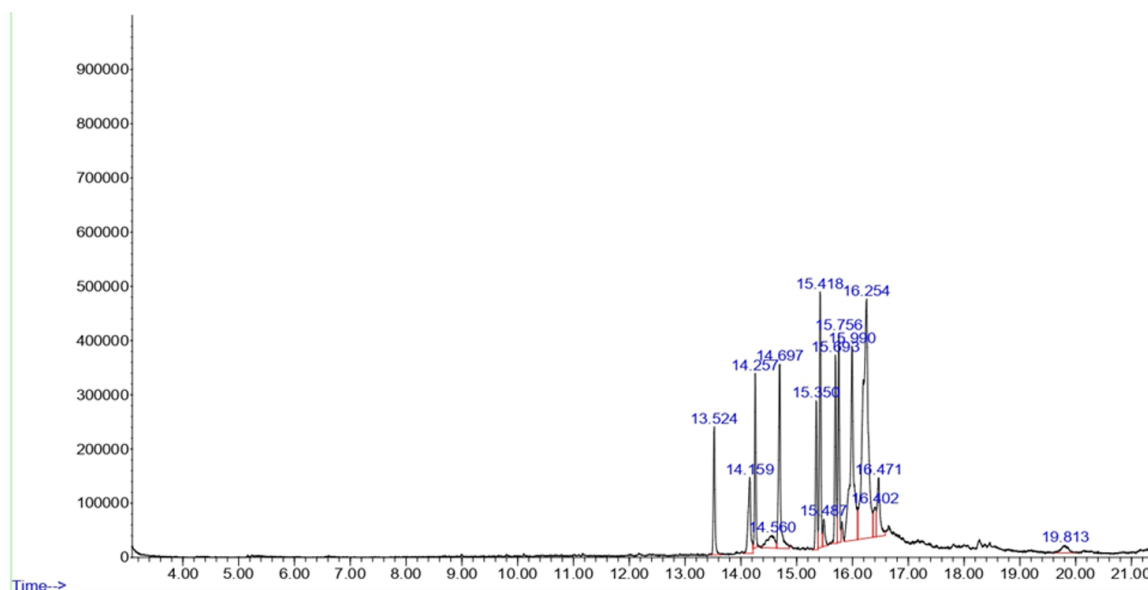


Figure 1. Chromatogram of the methanol extract of Cowpea without salicylic acid treatment.

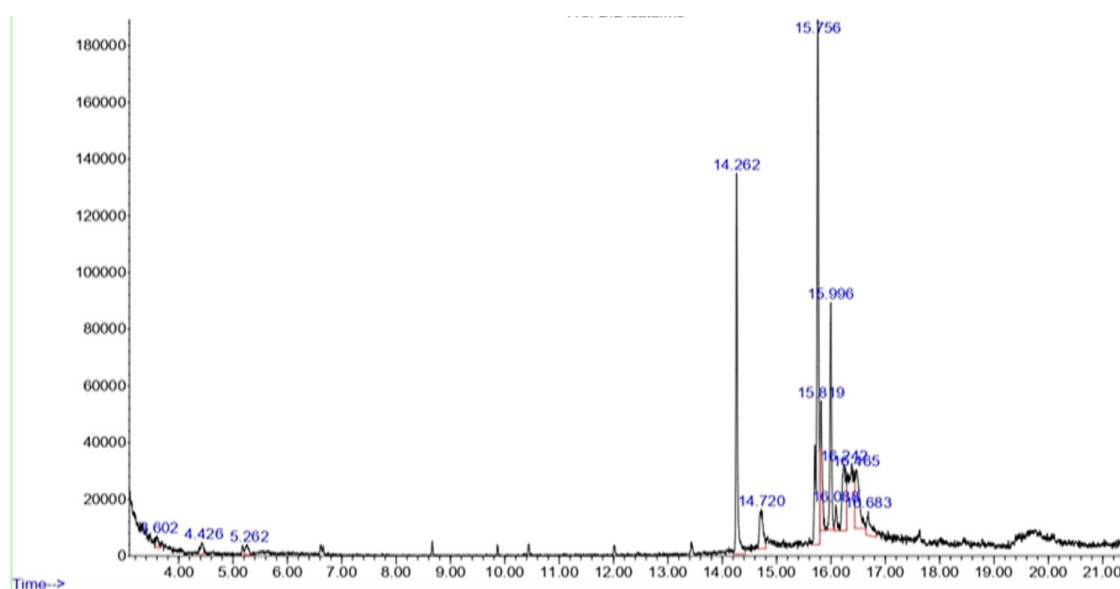


Figure 2. Chromatogram of methanol extracts of Cowpea treated with salicylic acid.

4. DISCUSSION

The results in Tables 1-2 showed the bioactive compounds obtained from the GC-MS analysis of the extract of cowpea treated with/without salicylic acid. The extracts of cowpea showed the presence of 43 bioactive compounds, whereas the extract of cowpea without salicylic acid showed 27 bioactive compounds. Salicylic acid is a plant bioregulator that had been shown to be implicated in various regulatory processes in plants, such as photosynthesis, nutrient uptake, stomatal regulation, and flowering, as well as playing a signaling role in the activation of disease resistance. It modulates cell division and expansion by regulating the expression of genes involved in the cell cycle and cell wall, and also stimulates the expression of these bioactive compounds, possibly through boosting antioxidant potential while neutralizing oxidant forms from free radicals generated when plants encounter stress. Overall, these bioactive compounds serve to defend plants and ensure the overall health of the plant, as well as support regulatory processes that enhance growth and development. Figures 1-2 showed the chromatogram of GC-MS analysis of the methanol extract of cowpea treated with/without salicylic acid, with each peak representing the presence of bioactive compounds at different retention times. This indicates that salicylic acid-treated cowpea

enhances and stimulates the expression of bioactive compounds as compared to the extract of cowpea without salicylic acid. This finding aligns with the work of Eshghi and Jamali [13], who reported stimulatory effects of salicylic acid on the total yield, number of fruits, and ascorbic acid content of strawberry. Thakur and Singh [41] reported that bioregulators such as gibberellic acid and ascorbic acid enhanced growth indices, leaf nutrient content (mineral nutrients), and total leaf chlorophyll content. Salas-Pérez et al. [42] also reported that the application of citric acid increases the quality and antioxidant capacity of lentil sprouts. Awika and Duodu [3] have also reported bioactive polyphenols and peptide constituents in cowpea (*Vigna unguiculata*) and their health-promoting properties. Hussain et al. [43] reported that foliar application of acetylsalicylic acid increased total carotenoids, total anthocyanins, reducing sugars, total soluble proteins, total flavonoids, and total phenolics contents of chickpea under drought stress. Asif et al. [44] also reported that plant-based zinc oxide, silicon, selenium, and amino-butyric acid, seaweed extracts, humic acids, and chitosan enhanced abiotic stress tolerance in crop plants by upregulating antioxidants and osmoprotectants, increasing the potential to synthesize phytoalexins and phenolics in response to stress, and by upregulating antioxidant enzymes such as superoxide dismutase and peroxidase activities, soluble protein, chlorophyll, and proline levels while decreasing malondialdehyde levels. All these suggest the stimulatory effects of bioregulators on plants. Similar work was also reported by Ashti et al. [11] that the GC-MS analysis of chickpea extract contains constituents of eugenol, beta-caryophyllene, caryophyllene oxide, methoxy acetic acid, and 3-tridecyl ester. Kim et al. [10] also reported the role of bioactive peptides found in soybeans as they relate to health functions because they are involved in nutritional functions by supplying amino acids, sensory functions, and bioregulatory functions in various physiological activities. Most importantly, they have a wide range of physiological functions, including serving as anti-inflammatory and anticancer agents, lowering blood pressure and serum cholesterol levels, improving immunity, and increasing calcium absorption. Elsherif et al. [12] reported that biogenic chlorapatite nanoparticles modulate antioxidant enzymes and increase non-enzymatic compounds (phenolics, flavonoids, ascorbic acid) in cowpea exposed to drought stress. Devi et al. [7] reported that bioregulators (salicylic acid and ethrel) significantly increased the growth, yield, and chemical constituents of soybean (*Glycine max*). Iswariya and Padmavathi [8] reported the impact of biostimulant seed treatment on cowpea seedling vigor. They discovered that treatment of cowpea with biostimulants (humic acid at a 5% concentration and brown kelp extract) can enhance seed germination, seedling growth, and biochemical parameters in cowpea var VBN4. Shukla et al. [9] reported that foliar application of NAA and maleic hydrazide significantly increased biochemical parameters (total phenol content, photosynthetic pigments, protein content, nitrate reductase activity) and yield attributes of chickpea (seed weight, number of pods). Sangeetha et al. [45] reported that GC-MS analysis reveals the presence of 2-piperidinone, catechol, benzofuran, 2,3-dihydro, high amounts of phenolic and flavonoid compounds when blackgram (*Vigna mungo* L.) and cowpea (*Vigna unguiculata* L.) were treated with aqueous leaf extracts from (*Mangifera indica* L.), (*Annona squamosa* L.), and moringa (*Moringa oleifera* L.). Elhefny and Abdelfattah [46] reported that GC-MS reveals volatile compounds (9,12-octadecadienoic acid, methyl ester, tigmasterol, and beta-sitosterol) in raw and cooked cowpea infested with *Callosbruchus maculatus*. Emmanuel and Cyril [47] reported that the dichloromethane extract of the seed of *Vigna unguiculata* contains secondary metabolites, including alkaloids, flavonoids, glycosides, phenols, saponins, sterols, triterpenoids, fatty acids, esters, alcohols, and ketones. Dinore et al. [48] reported that GC/MS chromatogram analysis of *V. unguiculata* pods reveals the presence of opioid α -N-Normethadol, a major constituent found in methanolic extract, and fatty acid esters; carotenoid is the second major constituent. LC/MS chromatogram and mass spectral analysis demonstrated the presence of flavonoids, carotenoids, and alkaloids as major phytochemicals, and the extracts were effective against *E. coli*, *S. pyogenes*, and *P. aeruginosa* with MIC 100 μ g/mL, similar to the standard ampicillin (MIC 100 μ g/mL). The pod extract showed significant DPPH scavenging activity, indicating its antioxidant potential. Kalim et al. [49] reported the presence of delphinidin 3-o-glucoside, chalcones, 2,4,4-trihydroxychalcone, dihydrochalcones, phloretin, dihydroflavonols, dihydroquercetin, hesperetin, neohesperidin, flavone (apigenin 7-o-

glucoside; apigenin 6-c-glucoside; hypolaetin; luteolin; vitexin, isovitexin), flavonols, kaempferol, kaempferol 3-o-rutinoside, kaempferitrin, quercetin in *Vigna radiata* (L.). Mmuta et al. [50] identified 53 bioactive compounds in 60-day-old Nigerian *Vigna radiata* leaves using GC-MS analysis, including steroids and 2,4-di-tert-butylphenol. All these findings revealed the nutraceutical properties of cowpea and the effects of bioregulators in upregulating these attributes.

5. CONCLUSION

Cowpea is an underutilized, nutritious, and affordable source of protein, though its role as a nutraceutical is underexplored due to technological constraints and low awareness. In the present study, Cowpea (Ife-BPC) seed, enhanced with 1.0 μ M of salicylic acid, modulates the expression of bioactive compounds with potential health benefits and can also help defend the plant during stress. Therefore, salicylic acid could be used to enhance bioactive compounds in plants for improvement in nutraceutical value and antioxidant potential.

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Competing Interests: The authors declare that they have no competing interests.

Authors' Contributions: Both authors contributed equally to the conception and design of the study. Both authors have read and agreed to the published version of the manuscript.

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