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Gas Chromatography Mass Spectrometry Based Metabolite Profiling In Ethanolic extract of Soybean (*Glycine max* (L.) Merr) seeds

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ABSTRACT
Multiple genetic, physiological, and environmental conditions are likely to alter the metabolic composition of plants. Metabolomics aims to provide a comprehensive and unbiased analysis of all metabolites and is therefore a very powerful tool for characterizing the plant metabolome and is of great interest to plant scientists. Because of the diversity of structural classes of metabolites, ranging from primary metabolites to very complex secondary metabolites, there is no single methodology that can measure the complete metabolome in one step. By employing a combination of different instrument platforms and techniques, the differences in the metabolite profiles can be revealed. In particular, gas chromatography–mass spectrometry (GC–MS). Current work provides an metabolite analysis of polarity based extracts of *Glycine max* (L.) Merr) seeds via UV-Vis. Spectroscopy and GC-MS analysis of one of the extract. Results showed that ethanolic extract had highest concnentration of 4',7-Dihydroxyisoflavone Daidzeol and GC- MS analysis of ethanolic extract showed that it contained 9,12-Octadecadienoic acid (Z,Z)-, methyl ester, 12-Octadecenoic acid, methyl ester, 11-Octadecenoic acid, methyl ester, Linoleic acid ethyl ester, (E)-9-Octadecenoic acid ethyl ester and 4',7-Dihydroxyisoflavone Daidzeol (whose retention time varied from 13. 797 min 9.097 min). These compounds can be isolated from the active extract and can be used for the evaluation of therapeutic effects via *in vitro* and *in vivo* studies.

Keywords: GC-MS, *Glycine max* (L.) Merr) seeds, ethanolic extract

INTRODUCTION

Soybean (*Glycine max* (*L.*) *Merr.*) is one of the most widely grown leguminous crops in the world grow in tropical, subtropical, and temperate climates and providing abundant protein and oil for human diet and animal feeding. Its seeds contain more than 36% protein, 30% carbohydrates, and appreciable amounts of dietary fiber, vitamins, and minerals. It also contains about 20% oil, which makes soybean the most important crop for edible oil production (Lim, 2012). Soya-based food products like tofu, soy milk, soy sauce, miso, etc. have been developed for human consumption while the oil extracted soya meal is used as a nutritious animal feed. Besides its use for domestic purposes, soy oil has multifarious uses in related industries for the production of pharmaceuticals, plastics, papers, inks, paints, varnishes, pesticides and cosmetics (Gupta, 2012).

Soybean contains numerous bioactive phytochemicals such as phenolic acids, flavonoids, isoflavones, saponins, phytosterols and sphingolipids and possesses excellent immune-active effects in the human body. The reported pharmacological properties of soy and its phytochemicals include antioxidant, estrogenic, antidiabetic, antihypercholesterolemic, antihyperlipidemic, antiobesity, antihypertensive, anticancer, antimutagenic, hepatoprotective, antiosteoporotic, antiviral, bifidogenic, antiinflammatory, immunomodulatory, neuroprotective, wound healing, antimicrobial, goitrogenic anti-skin aging, anti-photoaging activity and the effects of antinutritional factors (Lim, 2012).

To a large extent, these pharmacological attributes of soybean are attributable to the presence of isoflavones in soybean (Alghamdi et al., 2017). Due to importance of soybean and its products, it is necessary to investigate chemical composition across various genotypes. Therefore, this study was carried out to estimate the active ingredients including total phenolic acid content (TPC), total flavonoid content (TFC), protein content (PC) across 24 genotypes and Phytochemicals in methanolic extracts were evaluated using GC–MS.

Chromatographic techniques for the detection and identification of metabolites in plant material have undergone major changes in recent years due to improvements of analysis time, detection limit and separation characteristics. Depending on the biological question, one might distinguish between targeted and non-targeted strategies. Gas chromatography (GC) in particular is characterized by sensitivity and reliability of separations and detection of complex sample mixtures. Coupling with mass spectrometry (MS) provides highly robust analysis platforms compared to liquid chromatography (LC-MS) and allows for the identification of compounds based on the use of commercially or publicly available MS libraries and resources (Table 1) in combination with retention time index (RI) data (Jens Rohloff, 2015).

Table 1. Selection of freely software tools publicly available MS libraries and resources for structure elucidation and compound identification of GC-MS data for identification, deconvolution and alignment purposes (Rohloff, J. 2015).

Free GC/MS Analysis Software & Tools	
AMDIS—Automated Mass Spectral Deconvolution and Identification System	National Institute of Standards and Technology/Gaithersburg, MD, USA
Tagfinder—GC-MS analysis software (free upon request)	Max Planck Institute of Molecular Plant Physiology/Golm, Potsdam, Germany
MetaboliteDetector—Data deconvolution & analysis	TU Braunschweig, Germany
OpenChrom—Software for chromatography and MS	Dr. Philip Wenig/ Hamburg, Germany
Free GC/MS Alignment Tools	
Metalign—Processing of LC-MS and GC-MS data	Wageningen UR (University & Research centre)/Wageningen, The Netherlands
MZmine—Processing of LC-MS and GC-MS data	Turku Centre for Biotechnology/ Turku, Finland
MetaboAnalyst—Comprehensive tool suite for metabolomic data analysis	The Metabolomics Innovation Centre (TMIC)/University of Alberta, Canada
SpectConnect—GC-MS data alignment and analysis	Massachusetts Institute of Technology (MIT)/Boston, MA, USA

MATERIALS AND METHODS

STUDY DESIGN

It is an experimental study, conducted at Institute of Molecular Biology and Biotechnology, The University of Lahore, Lahore, Pakistan Council for Scientific and Industrial Research (PCSIR), Lahore and Chughtai lab, Lahore, Punjab, Pakistan.

COLLECTION AND IDENTIFICATION OF PLANT MATERIAL

Glycine max (L.) seeds were obtained from Nawab Shah, Sindh region of Pakistan, identified by expert taxonomist at Government College University, Lahore with botanical number of GC.Herb.Bot.3779.

PREPARATION OF EXTRACTS

Fresh seeds were shade dried at room temperature grind, followed by grinding of dried leaves into powdered form (80 mesh) by mechanical means, added n-hexane (1:10 ratio) and kept for shaking for 24 hours in shaker incubator (K-J-201BD), followed by the centrifugation for 15 minutes at 5000 rpm (SIGMA 203,43191) and filtration through Whatman filter paper 1.0. Filtrate had been shade dried at room temperature while next solvent (ethyl acetate, ethanol, methanol and dist. water respectively) has been added in residue with repetition of previous procedure. Dried filtrate has been re- dissolved in 15 % DMSO to prepare stock solution (1.0 mg/ ml) (Tahir et al. , 2023, Waris et al. , 2023 and Khan et al. , 2023).

UV-VISIBLE SPECTROSCOPY

A double beam UV-visible spectrometer (UV-530, Jasco) with spectra manager software was used for the analysis. Quartz cells having a 3 cm length with 1 cm path length were used for spectral measurement. Weighing balance (Essae, Vibra HT) with internal calibration mode was used for the accurate weighing purpose. Accurately weighed 5 mg of daidzein was transferred into the calibrated volumetric flask and dissolved in 5 mL mixture of ethanol and water (50:50 v/v) to achieve a stock solution of 1000 µg/mL (Stock-I). Further diluting Stock- I solution with a co-solvent system of ethanol and water to get the desired concentration of 10 µg/mL (Stock II). Stock-II solution was scanned using full scan mode for the entire range of UV and visible i.e. 800 to 200 nm with a co-solvent system of ethanol and water as a blank. After obtaining the spectrum, λ_{max} was identified with the help of software. To achieve reproducible results, the above method was repeated five times. The calibration curve was prepared by diluting the stockII solution to achieve the six different calibration standards representing 6.25, 12.50, 18.75, 25.0, 31.25, 62.5 µg/mL strength. The absorbance of each calibration standard was measured at pre-identified λ_{max}; 248 nm using fixed wavelength measurement mode. The calibration curve representing concentration vs. absorbance was plotted Above mentioned procedure was repeated five times so that reproducible results can be obtained (Bhusari et al. , 2020, Zahid et al. , 2021 and Ahmed et al. , 2018).

GAS CHROMATOGRAPHY-MASS SPECTROMETRY (GC-MS) ANALYSIS

It was performed on the freeze-dried extracts at Chughtai Lab in Lahore, Pakistan, using ethanol as the solvent. The principal aim of the analysis was to identify any chemicals contained in the extract that might possess anticancer effects. Adhering closely to international standards for chemical analysis, Chughtai Lab is certified by ISO-15189 and The College of American Pathologists (CAP). The Tox-Analyzer system was used for chemical identification and quantification for the GC-MS study. One milliliter per minute of 99.99% pure helium was used as the carrier gas.The oven temperature was set to begin at 60°C and rise steadily at a rate of 10°C per minute until it reached 310°C in 4 minutes. The injector temperature was maintained at 250°C. To identify chemicals, the acquired spectrum data were compared to the NIST20.L and SWGDRUG 3.9.L libraries, each of which had a minimum quality threshold of 60 and 50, respectively. With the use of the total ionic chromatogram's

(TIC) peak area expression, the relative percentage levels of each component were found. The purpose of this thorough investigation is to find new substances in the probiotic extract that may have anticancer qualities.
Using the protocol described here facilitates routine determination of the relative levels of 300–500 analytes of polar and nonpolar extracts in ~400 experimental samples per week per machine.

DATA ANALYSIS

The data were analyzed for descriptive statistics (mean, standard deviation, coefficient of variability, minimum and maximum values) and principal component analysis using statistical software Past3 program (Hammer et al., 2001).

RESULTS

The identification of the wavelength of maximum absorbance is a prerequisite for quantitative UV analysis. Solution representing absorbance value less than 1 is generally considered to be suitable for the determination of the wavelength of maximum absorbance. Considering the prerequisite and the suitability, the determination of maximum wavelength for the daidzein solution was carried out using a full scan mode of UV-Visible spectrophotometer (figure 1). A full scan was processed using UV software and the λmax was identified with the help of software. It was found to be 248 nm for daidzein. *The concnentration of daidzein was found to be 7.05 mg/ 100 mL in polarity based ethanolic extract* while it was 5.02 mg/ 100 mL in pure ethanolic extract *of G. max* (L.) Merr) seeds.
Quantification of unknown samples by UV-Visible spectrophotometer or any other instrumental method of analysis needs a reproducible calibration curve and an equation stating the correlation between concentration and the response. As compared to the graphical method, the above-stated method is widely accepted and reproducible. Considering the utility of the quantitative analysis of daidzein, the calibration curve for daidzein was developed using six different calibration standards. The absorbance of different calibration standards at 284 nm was recorded using a fixed wavelength mode of UVVisible spectrophotometer. The calibration curve was repeated five times and the mean values ± deviation was reported as shown in Table 1.

Table 1 Calibration standard data for daidzein (4',7-Dihydroxyisoflavone Daidzeol)

Concentrations (µg/ mL)	Absorbance
6.25	***0.265***
12.50	***0.881***
18.75	***1.613***
25.0	***2.248***
31.25	***3.126***
62.5	***4.06***

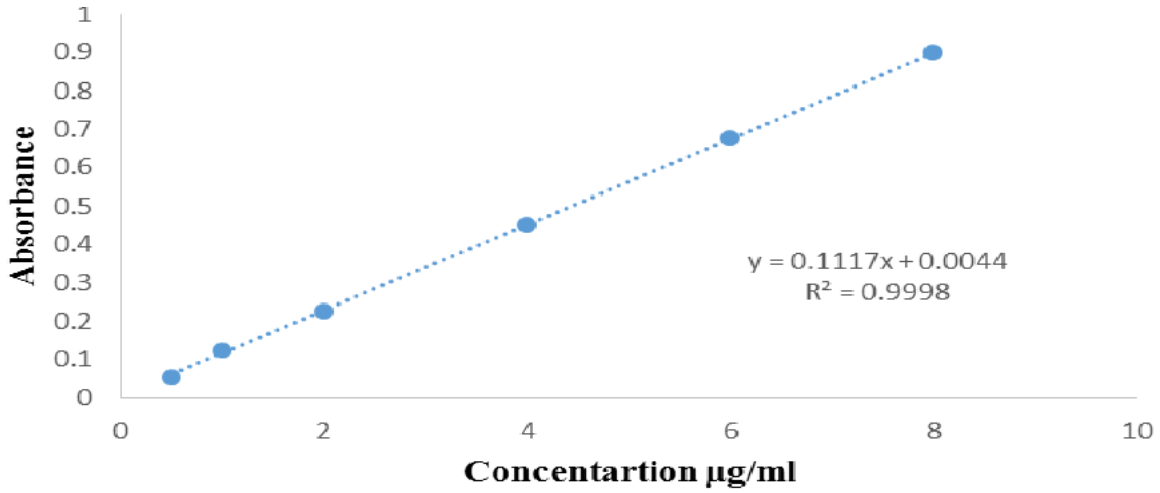
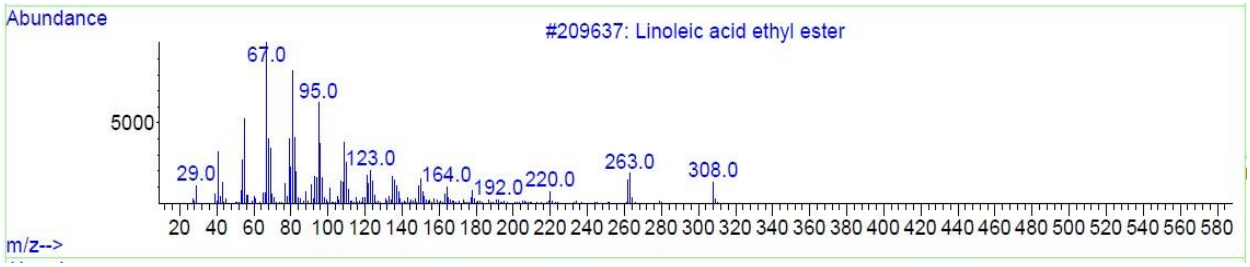


Figure 1 Calibration cure for daidzein (4',7-Dihydroxyisoflavone Daidzeol)

GC–MS ANALYSIS

A large number of phytochemical were identified in the ethanolic extract of soybean seeds using GC–MS analysis. A total of 08 compounds were identified based on peak area, retention time and molecular formula. A large number of bioactive phytochemicals including phenolic acids, flavonoids, isoflavones, saponins, phytosterols and sphingolipids were also reported previously for soybean (Luthria et al., 2007, Lee et al., 2008). The first compound identified at retention time of 13. 797 min was (E)-9-Octadecenoic acid ethyl ester, whereas 4',7-Dihydroxyisoflavone Daidzeol was the last compound which with longest retention time (9.097 min) (Table 2). There were wide variation in the compositions of phytochemicals in single extract and are shown in Table 2, along with their biological activities. The phytocompounds identified belonged to esters and isoflavones.


Abundance
#209637: Linoleic acid ethyl ester
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95.0
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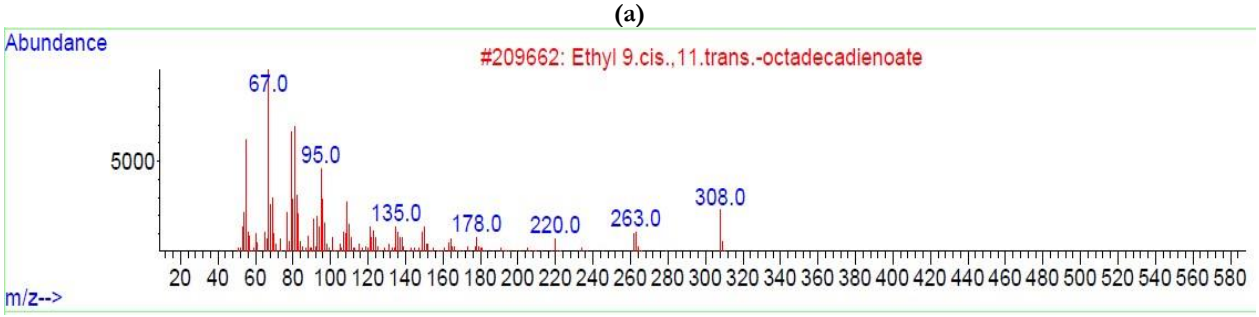
29.0
123.0
164.0
192.0
220.0
263.0
308.0
m/z-->

(a)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#209662: Ethyl 9.cis.,11.trans.-octadecadienoate
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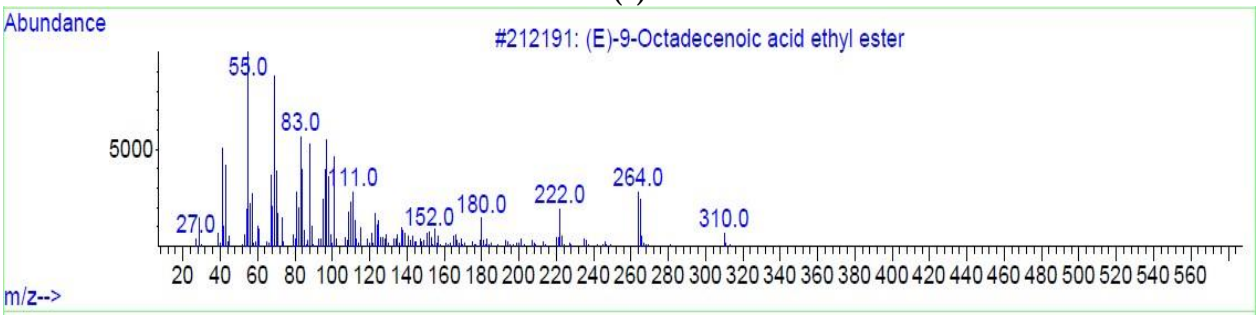
308.0
m/z-->

(b)


Abundance
#212191: (E)-9-Octadecenoic acid ethyl ester
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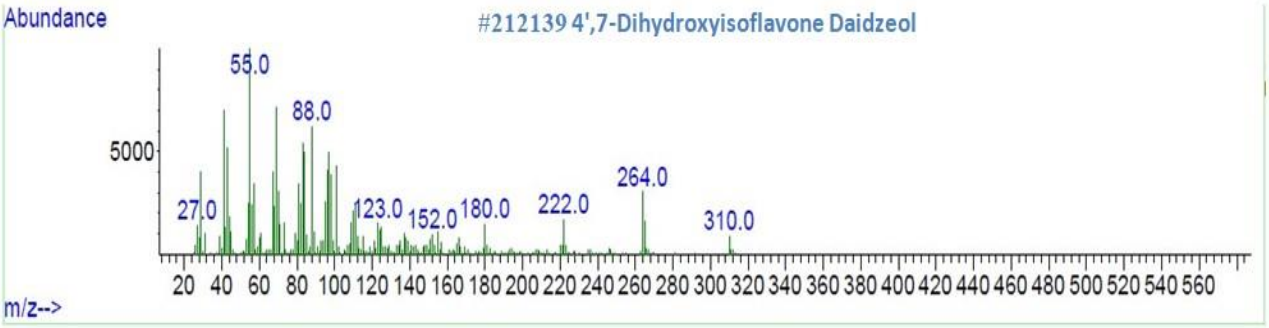
111.0
264.0
27.0
152.0
180.0
222.0
310.0
m/z-->

(c)


Abundance
#212139 4',7-Dihydroxyisoflavone Daidzeol
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5000
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27.0
123.0
152.0
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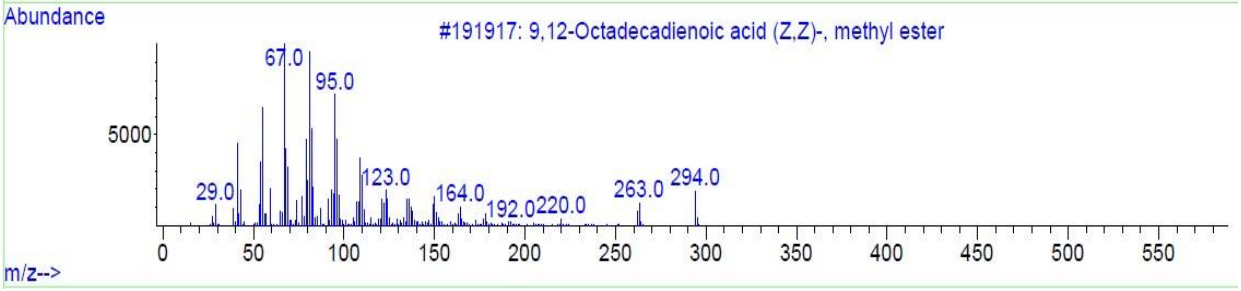
222.0
310.0
m/z-->

(d)


Abundance
#191917: 9,12-Octadecadienoic acid (Z,Z)-, methyl ester
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29.0
123.0
164.0
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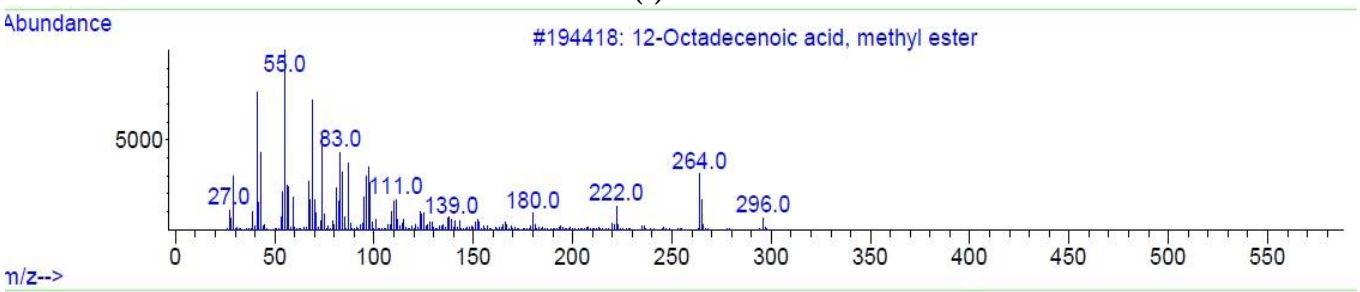
220.0
263.0
294.0
m/z-->

(e)


Abundance
#194418: 12-Octadecenoic acid, methyl ester
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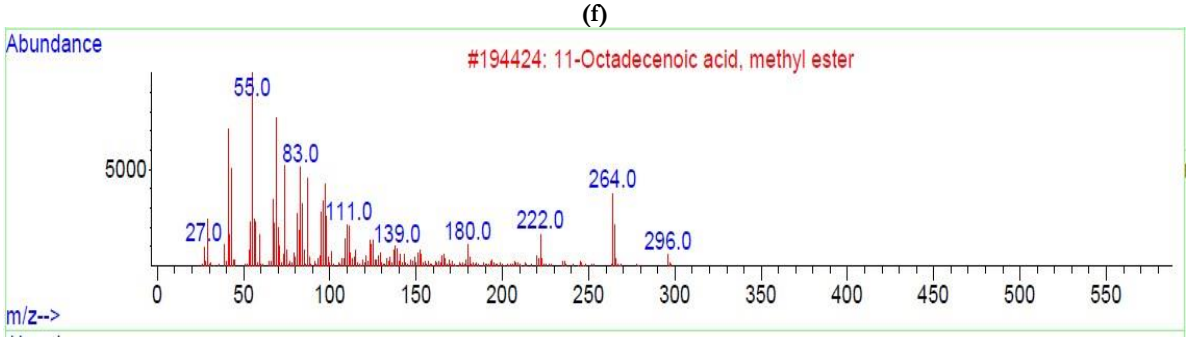
264.0
27.0
111.0
139.0
180.0
222.0
296.0
m/z-->

(f)



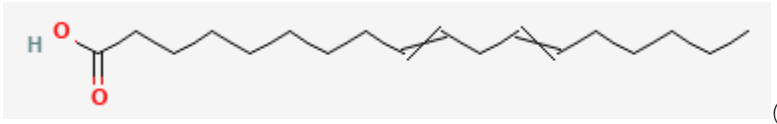
(g)

Figure 2 (a- g) Chromatograms of GC-MS Analysis for ethanolic Extract of *G. max* (L.) Merr) seeds: Identifying Anticancer Compounds. The GC-MS chromatogram of the G. max seed extract revealed multiple peaks, indicating the presence of various compounds. Each peak corresponds to a specific compound that was identified based on its retention time (RT) and mass spectral data. The key peaks of interest were analyzed and the compounds were identified with their respective area percentages and quality scores. The major and minor components were listed in the table below.

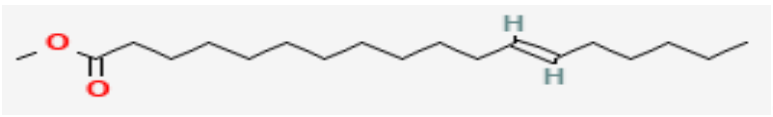
Table 2 GC-MS Identified Anticancer Compounds from ethanolic extract of *G. max* (L.) Merr.) seed

Pk#	gRT	Area%	Compound	Figures	Molecular Forumla and molecular weight	CAS#	Nature	Activity
6	12.437	2.39	9,12-Octadecadienoic acid (Z,Z)-, methyl ester	Figure 3 (a)	● $C_{18}H_{32}O_2$ 280.4 g/mol	000112-63-0	Ester	Anticancer
7	12.552	4.55	12-Octadecenoic acid, methyl ester	Figure 3 (b)	● $C_{18}H_{32}O_2$ 280.4 g/mol	056554-46-2	Ester	Anticancer
7	12.552	4.55	11-Octadecenoic acid, methyl ester	Figure 3 (c)	● $C_{19}H_{36}O_2$ 296.5 g/mol	052380-33-3	Ester	Anti-inflammatory
8	12.661	1.00	11-Octadecenoic acid, methyl ester	Figure 3 (c)	● $C_{19}H_{36}O_2$ 296.5 g/mol	052380-33-3	Ester	Anti-inflammatory
11	13.658	22.20	Linoleic acid ethyl ester	Figure 3 (d)	● $C_{20}H_{36}O_2$ 308.5 g/mol	000544-35-4	Ester	Anticancer
12	13.797	13.07	(E)-9-Octadecenoic acid ethyl ester	Figure 3 (e)	● $C_{20}H_{38}O_2$ 310.5 g/mol	006114-18-7	Ester	Anticancer
9	9.097	13.07	4',7-Dihydroxyisoflavone Daidzeol	Figure 3 (f)	● $C_{15}H_{10}O_4$ 254.24 g/mol	005221-19-8	Isoflavone	Anticancer

This table lists the anticancer compounds identified from *G. max* (L.) Merr) seeds extracts through GC-MS analysis. It includes information on the retention time (RT), the percentage of the area under the peak (Area%), the compound name, reference number (Ref#), CAS number (CAS#), and quality score (Qual).



(a)



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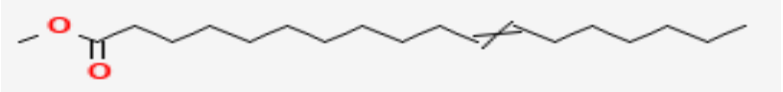
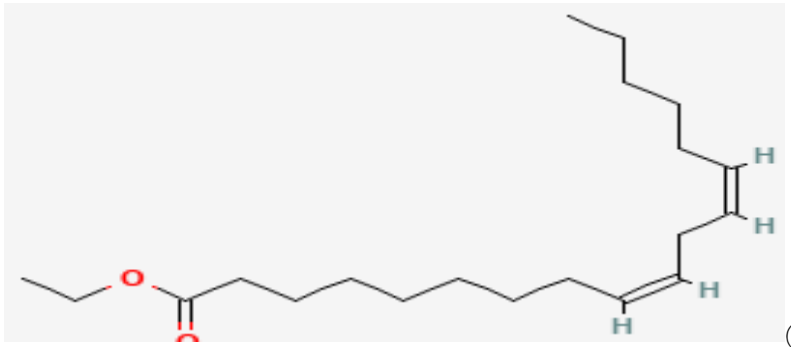
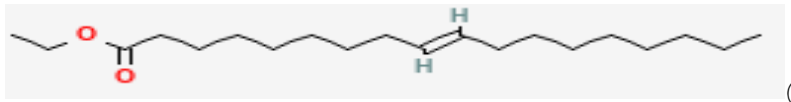


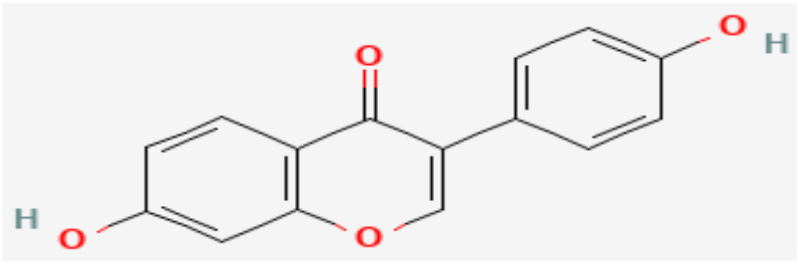
(c)



(d)



(e)



(f)

Figure 3 (a- f) Structures of identified compounds in ethanolic extract of *G. max* (L.) Merr.) seeds

Noteworthy compounds with potential anticancer properties are:

- Linoleic acid ethyl ester (22.20% Area)
- (E)-9-Octadecenoic acid ethyl ester (13.07% Area)
- 12-Octadecenoic acid, methyl ester (4.55% Area)
- 4',7-Dihydroxyisoflavone Daidzeol (8.01 % Area)

These compounds are significant due to their high area percentages, indicating a substantial presence in the extract, which suggests potential efficacy in anticancer activity.

GC–MS analyses revealed that the ethanolic extract is predominantly composed of one heterocyclic compound and esters. Only one isoflavone has been detected in this extract while rest of all compounds are esters. These phytochemicals are responsible for various pharmacological actions like antimicrobial and antioxidants activities (Tapiero et al., 2002). The phytochemicals identified through GC–MS analysis proved to be active in many biological activities. Phytochemicals have been reported to possess potent antioxidant and anti-cancer and anti-inflammatory activities to a greater or lesser extent and play a vital role in plant metabolism (Wei et al., 1999).

DISCUSSION

Aldehydes are reported to possess powerful antimicrobial activity because of their highly electronegative arrangement of an aldehyde group conjugated to a carbon to carbon double bond (Moleyar and Narasimham, 1986), suggesting an increase in electronegativity increases the antibacterial activities in those genotypes (Kurita et al., 1979, Kurita et al., 1981). Such electronegative compounds react with vital nitrogen components, e.g. proteins and nucleic acids and consequently inhibit microorganisms.

The genotype 3803 and Indo-11 of *G. max* (L.) Merr. recorded the 08 number of ketonic compounds, followed by Giza 35 and USA-1 genotypes having six (6) ketonic compounds each. Ketones might be formed by beta-oxidation of fatty acids, which generated a few important flavor compounds (Yu et al., 2008).

Volatile compounds are also formed through fatty acid metabolism, producing alcohols, acids, and esters. Most alcohols are derived from bioremediation of unsaturated fatty acids, and are also a prerequisite for the formation of long-chain esters. Many alcoholic compounds like 4-methyl-2-haptanol, 1-undecanol alcohol, 1,2,3-Propanetriol, Isosorbide (D-Glucitol, 1,4,3,6-dianhydro) and 1,3-Dioxolane-4-methanol (Glycerol formal) were detected among the tested genotypes. 4-methyl-2-haptanol was detected in one genotype (Giza 35) while 1,2,3-Propanetriol was present in nine genotype and Isosorbide was appeared in three genotype (Saegeman et al., 2008).

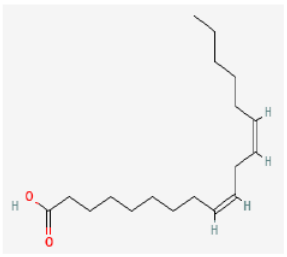
Although a total of 07 esters, namely 9,12-Octadecadienoic acid (Z,Z)-, methyl ester, 2 peaks of 12-Octadecenoic acid, methyl ester, 2 peaks of 11-Octadecenoic acid, methyl ester, Linoleic acid ethyl ester, (E)-9-Octadecenoic acid ethyl ester were identified in ethanolic extract of soybean while genotype A-1 contained maximum 6 esters compounds followed by others genotypes of soybean (Romal-1, Giza 83, Clark, 3803 and Argentinian) having five (5) esters compounds (Priya and Vijaylakshmi, 2011).

Multiple phytocompounds in the form of lipids, isoflavones, carbohydrates and proteins have been isolated from previously available literature (Table 3) and multiple *in silico* and *in vivo* studies have proven that these compounds have cured PCOS in different animal models

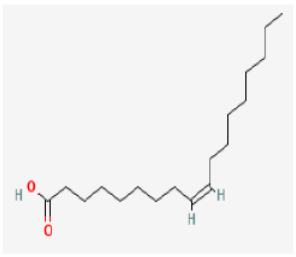
Table 3 Phytocompounds and their properties in polarity based extracts of *G.max* (L.) (Waris et al. , 2023)

Compounds	Quantity	Molecular formula	Molecular weight (g/mol)	Structures (Figure 4)	PubChem I.D	Solubility
1-Palmitoyl-2-linoleoylphosphatidyl choline	2-3%	$C_{42}H_{80}NO_8P$	758.1	(a)	5287971	polar/ non-polar, soluble
Linoleic acid	48-58%	$C_{18}H_{32}O_2$	280.4	(b)	5280450	non-polar, soluble
Oleic acid	17-30%	$C_{18}H_{34}O_2$	282.5	(c)	445639	non-polar, soluble
Palmitic acid	9-13%	$C_{16}H_2O_2$	256.42	(d)	985	non-polar, soluble
Linoleic acid	5-11%	$C_{18}H_{30}O_2$	278.4	(e)	5280934	non-polar, soluble
Stearic acid	3-5%	$C_{18}H_{36}O_2$	284.5	(f)	5281	non-polar, soluble
Campesterol	19-23%	$C_{28}H_{48}O$	400.7	(g)	173183	non-polar soluble
Stigma sterol	17-19%	$C_{29}H_{48}O$	412.7	(h)	5280794	non-polar, soluble
gamma-Tocopherol	44-60%)	$C_{28}H_{48}O_2$	416.7	(i)	14986	non-polar, soluble
Daidzin	67–516 µg/g	$C_{21}H_{20}O_9$	416.4	(j)	107971	non-polar, soluble
Genistin	91–1079 µg/g	$C_{21}H_{20}O_{10}$	432.4	(k)	5281377	non-polar, soluble
Glycitin	12–177 µg/g	$C_{22}H_{22}O_{10}$	446.4	(l)	187808	non-polar, soluble
Malonyldaidzin	217–768 µg/g	$C_{24}H_{22}O_{12}$	502.4	(m)	9913968	non-polar, soluble
Malonylglycitin	43–158 µg/g	$C_{25}H_{24}O_{13}$	532.4	(n)	23724657	non-polar, soluble
Malonylgenistin	64–2446 µg/g	$C_{24}H_{22}O_{13}$	518.4	(o)	15934091	non-polar, soluble
Apigenin	4.3–265 µg/g	$C_{15}H_{10}O_5$	270.24	(p)	5280961	non-polar, soluble
As insoluble fibers	15-35%					polar soluble
As essential amino acids	35-40%					polar /non-polar, soluble

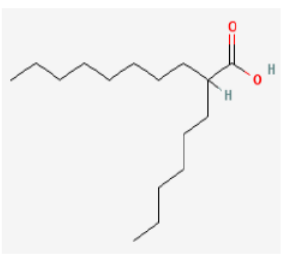
(a)



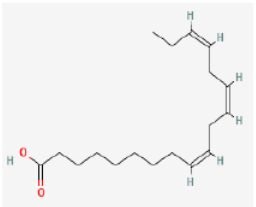
(b)



(c)

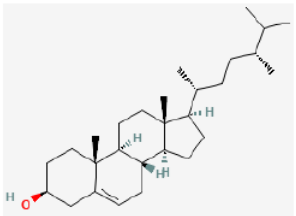


(d)

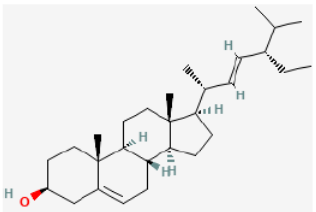


(e)

(f)



(g)



(h)

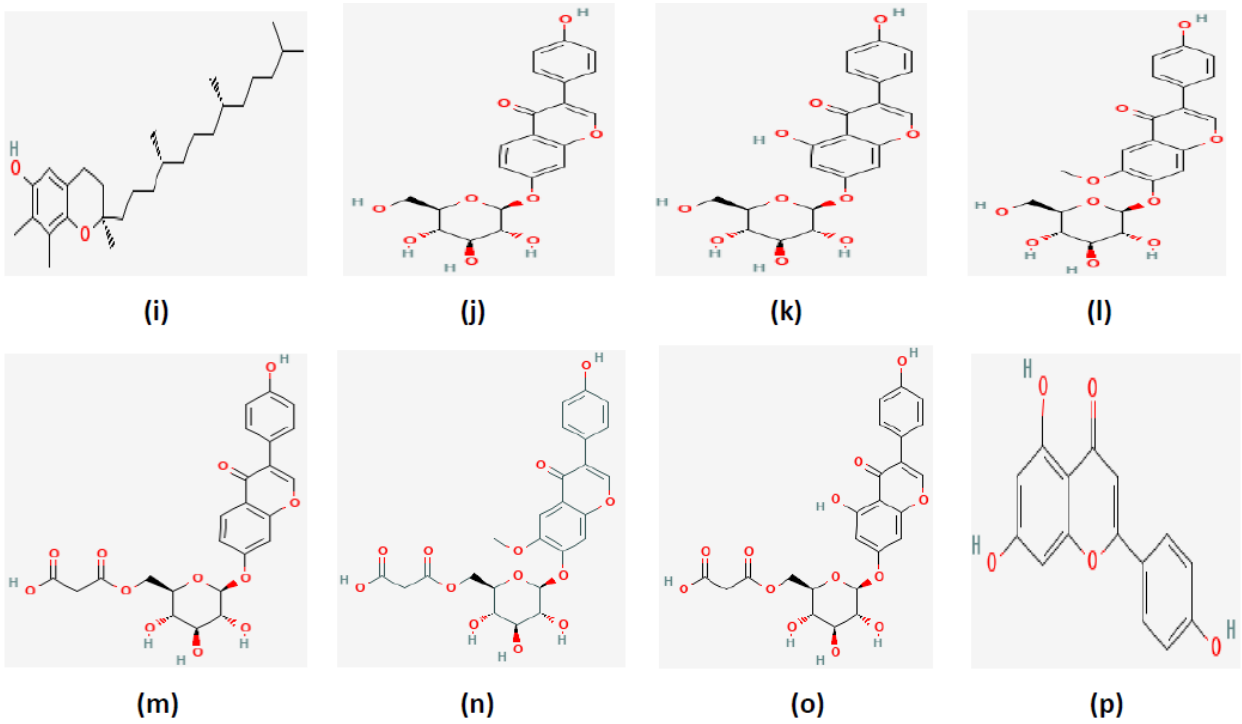


Figure 4Structures of Phytocompounds Found in *G.max*.L. Seeds (Waris et al. , 2023)

Taken together, some of the detected compounds are reported to be potential therapeutic agents. Moreover, antimicrobial, antioxidant and anti-inflammatory activities were demonstrated by most compounds in this study indicating that different compounds can display similar activity and this could be due to presence of similar functional groups. The presence of various antioxidant and anti-inflammatory compounds may be the reason for the presence of antioxidant properties of soybean methanolic seed extract (Yang et al., 2001, Hedlund et al., 2003).

Soybeans are rich in proteins and lipids and have become a staple part of the human diet. Besides their nutritional excellence, they have also been shown to contain various functional components, including isoflavones, and have consequently received increasing attention as a functional food item. Isoflavones are structurally similar to 17-β-estradiol and bind to estrogen receptors (ERα and ERβ) (Wang et al. , 2017). The estrogenic activity of isoflavones ranges from a hundredth to a thousandth of that of estrogen itself. Isoflavones play a role in regulating the effects of estrogen in the human body, depending on the situation. Thus, when estrogen is insufficient, isoflavones perform the functions of estrogen, and when estrogen is excessive, isoflavones block the estrogen receptors to which estrogen binds, thus acting as an estrogen antagonist. (Sukumaran et al. , 2018). In particular, estrogen antagonistic activity is important in the breast, endometrium, and prostate, and such antagonistic activity suppresses cancer occurrence. S-Equol, a metabolite of genistein and daidzein, has strong antioxidative effects; however, the ability to metabolize daidzein into S-equol varies based on racial and individual differences. The antioxidant activity of esters and isoflavones may be effective in preventing dementia. Daidzein and genistein also reduces allergic responses by limiting the expression of mast cell IgE receptors (Lu et al. , 2018). In addition, they have been known to prevent and treat cardiovascular diseases, metabolic syndromes, osteoporosis, diabetes, brain-related diseases, high blood pressure, hyperlipidemia, obesity, and inflammation. Further, it also has positive effects on menstrual irregularity in non-menopausal women and relieving menopausal symptoms in middle-aged women (Kim I. S. 2021).

ß -catenin is the key mediator of the Canonical Wnt pathway. In the absence of a Wnt ligand, ß-catenin is degraded by a destruction complex. The main components of this complex include AXIN, adenomatous polyposis coli (APC), casein kinase 1 (CK1), and glycogen synthase kinase 3ß (GSK3ß), as well as the E3 ligase, ßTrCP. Protein phosphatase 2A (PP2A) is also associated with the ß-catenin destruction complex. AXIN is a scaffolding protein that has interaction sites for multiple proteins including PP2A, APC, GSK3ß, and CK1. The most common genetic alteration in the Wnt/ß-catenin pathway involved in EOC is in the ß-catenin gene, CTNNB1. In EOCs a missense mutation in CTNNB1 was always found within the amino terminal domain. Soybeans (*G. max* L.), has been reported as the main source of isoflavones. Phytoestrogen properties of soy isoflavones showed their activity as ligands for estrogen receptors and exhibited the estrogenic potency as reported in the previous *in vitro* and *in vivo* studies. The major functional components include Carbohydrates, fats, Proteins, Saponins, Lecithin, Linolenic acid, Linoleic acid, Phytosterols and Isoflavones. Due to these valuable constituents, it possesses multiple therapeutic activities. Current in-silico study showed that Daidzein from *G. max* L. has maximum binding affinity with beta catenin, which may be ultimately inhibited followed by the inactivation of Wnt/β -Catenin pathway. Moreover, it has been shown to relive sleep disorders, may help managing diabetes, prevents osteoporosis, improves blood circulation and provide good care of pregnancy (Hafeez et al. , 2024).

Out of all soybean compounds, isoflavones have female estrogen like effects and can bind to estrogen receptors. They can regulate metabolic and endocrinal disorders. They also have the effect of aromatase inhibition so they can affect the transformation of androgens to estrogens and decrease hyperandrogenemia in PCOS patients. This decrease of hyperandrogenemia by non-chemical source can be beneficial for the PCOS patients (Farkhad et al. , 2019). Many studies in

past were conducted to highlight the role of *G.max* (L) in hormonal and histological improvement of PCOS, both in animals and humans. A recent study observed the role of soy bean in normalizing of abnormality in hormonal profile in PCOS, through acting anti-inflammatory pathways (Kim et al. , 2021). Another new study also identifies the same about G.max (L) in PCOS animals (Abubakar et al. , 2020). Current study is helpful for further isolation of active compounds in *G.max* (L.) that can be used by pharmaceutical companies to cure PCOS.

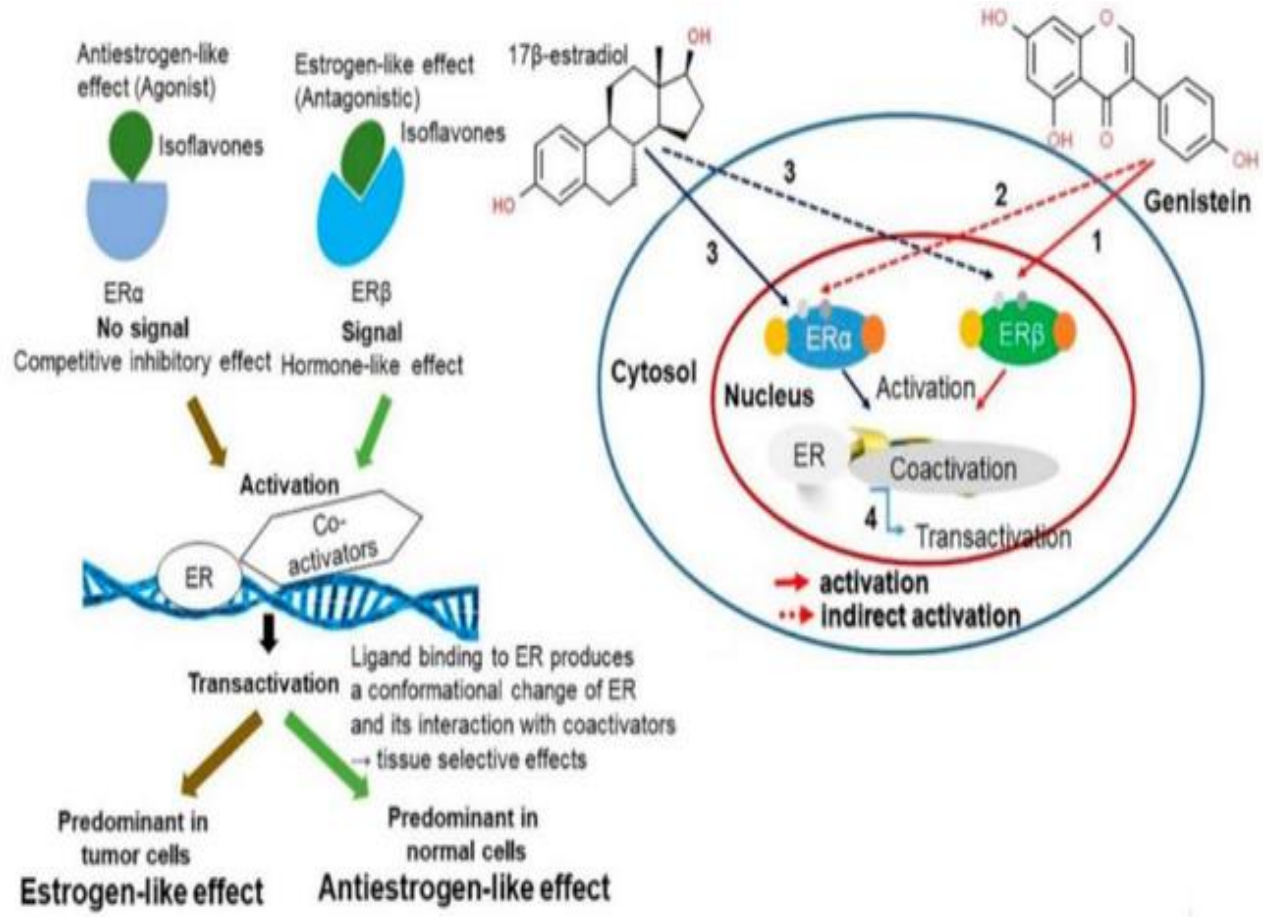


Figure 4Phyto- Estrogenic Activity of Soybean Isoflavone through Estrogen Receptor Beta (Erβ) Predicted Action Mode of 17β-Estradiol. Figure adapted from Ma et al. , (2021) with following steps:

- Preferential interaction with ERβ to Activation of ERβ-dependent mechanism
- Binding as an agonist to ERα to Activation of conventional ERα- mediated action mode
- Competing with 17β-estradiol to bind to ERα and ERβ to Playing as functional antagonist
- Ligand interacting with ER affects three-dimensional structures of ER and it's binding to co-activators to Tissue-specific effects.

CONCLUSION

Spectrophotometric analysis showed that among all polarity based extracts, ethanolic extract have maximum amount of diadzein (this compound was reported to be imported anticancer compound against ovarian cancer) that's why ethanolic extract was screened for its GC-MS analysis which proved that overall seven esters and one isoflavone were present in screened extract.

Ethical Approval: Not applicable

Supplementary Materials: Not Applicable

Author Contributions: "Conceptualization and validation: A. A.; methodology, S. H.; formal analysis, S.H. investigation, A.A. and S.H; resources, A.A and G. R.; writing, review and editing, A.A; supervision. All authors reviewed and commented on the manuscript.

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Institutional Review Board Statement: Not applicable

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