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A COMPARATIVE STUDY OF AQUEOUS EXTRACT AND COLD PRESS OIL OF GRAPE SEED

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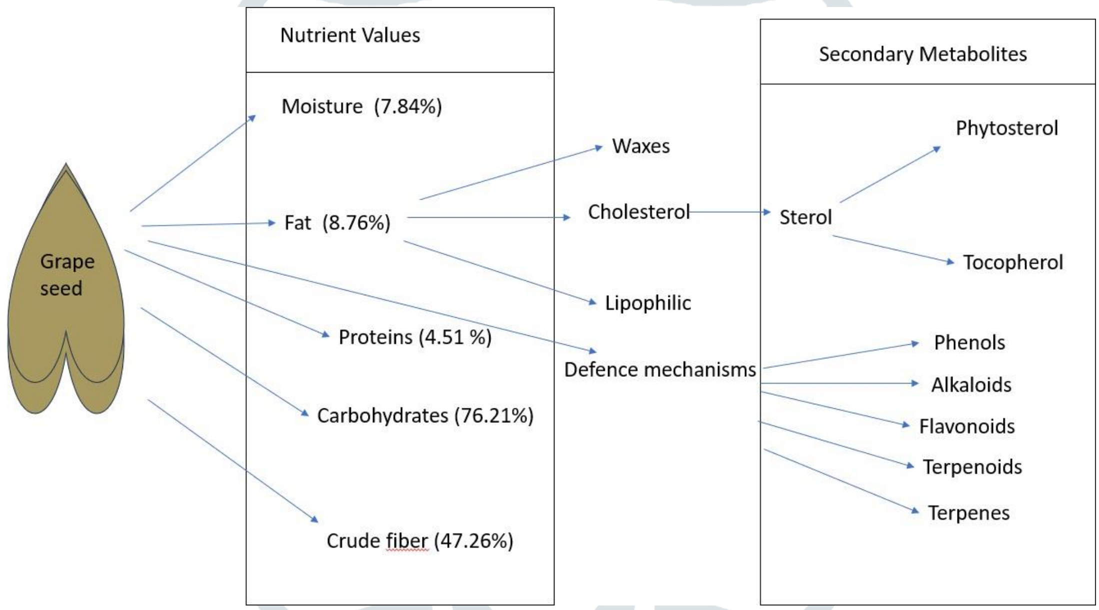
Introduction

Grapes are recognized as one of the most valuable fruits in worldwide, approximately 60 million tonnes production form China, USA, Turkey and Italy in a year *(Ma & Zhang, 2017)*.China emerging as the predominant cultivator by 2024.India is seventh largest producerin the world. The primary cultivators in India include Maharashtra (80%) (Nasik, Solapur, Sangli, and Pune districts), followed by northern Karnataka (Bijapur, Belgaum, Kadappa, Bagalkot districts), Andhra Pradesh (Rangareddy district), and Tamil Nadu (Krishnagiri, Theniand Coimbatore districts). It grown in sub-tropical climate where temperature range between (25-30)ºc *(Mitra, 2018)* boast an 8,000-year legacy, predominantly utilized for winemaking and as dried fruit *(Inglis, 2022)*. The 10-12 kg of seeds was present in 100 kg in the fruit *(Ma & Zhang, 2017; Shivananda Nayak et al., 2011)*. Nowadays, seeds are not used for germinationand wine industries considered as a natural waste product .They has served potent source of nutrients, particularly their seeds, which contain lower quanity of an oil present in the inner layer of endosperm and embryo, accounting for approximately 8.76% of the composition. TheWorld Health Organization (WHO) record show that 80% of the global population followingthe natural remedies for health issues *(Manjamalai & Grace, 2012)*. Traditional medicine, alsoreferred to as indigenous or folk medicine, encapsulates accumulated medical knowledge and practices developed across diverse cultures over generations. This form of medicine employs a broad spectrum of health practices, beliefs, and therapies, including plant, animal, and mineral-based medicines, spiritual therapies, manual techniques, and exercises, applied eitherindividually or in combination to promote well-being, as well as to treat, diagnose, or preventillnesses. Predominantly, plants and their oils serve as natural therapies *(Eshete & Molla, 2021;Karunamoorthi et al., 2012; Mafimisebi & Oguntade, 2010; Oreagba et al., 2011)*. The oil extracted from plants in non-conventional method , known as “Cold press oil”, has derived from break down cholesterol. The oils are highly concentrated hydrophobic liquids containingvolatile and non-volatile compounds with aromatic properties. Commercially cultivated plantseeds, utilized in traditional medicine, are prized for their pharmacological advantages and grown for both medicinal and economic gains. *(Mafimisebi & Oguntade, 2010)*.These seeds harbour active secondary metabolites enhancing their health-promoting attributes and are frequently employed in the form of oils, extracts, or directly as seeds in various traditional remedies. It is crucial to utilize these seeds and their derivatives judiciously and under expert guidance, particularly when applied for health purposes. However, they possess high medicinalvalue and are used for biological studies .In recent years grape seeds extract has become the popular on the on the market as nutritional supplement especially in the Australia, Korea, Japan and USA(Ma & Zhang, 2017) . In our present investigation, we have analyzed the antioxidant potency for GSAE (Grape Seed Aqueous Extract) and GSO (Grape Seed Oil).

Figure 1: Nutrient and Secondary metabolites present Grape seed



Materials And Methods

2.1 Collection of Plant

The plant specimen *Vitis vinifera***.** L (Grape) was collected form a farm in Madampatti Coimbatore, Tamil Nadu, India. The plant materials were taxonomically identified and authenticated by the Botanical Survey of India **BSI/SRC/5/23/2022/Tec/501.**

2.2 Extraction of GSAE and GSO

2.2.1 Aqueous Extraction (GSAE) :

The dried seeds material was initially crushed using a grinder, resulting in a fine powder. Subsequently, 300g of this powdered material was mixed with distilled water and subjected toSoxhlet extraction for 6 hours, utilizing 600 mL of distilled water as the solvent*(Al-Otibi et al.,2021*). Subsequently dried in a hot air oven until a dark brown powdered form was obtained.

2.2.2 Oil Extraction (GSO):

The dried seeds material underwent crushing using a wooden impeller, resulting in a finely powdered form. Subsequently, 15,000g of this powder was mixed with distilled water and processed using a Cold press machine for a of 6 hours, utilizing 1L of distilled water as the solvent. The extraction process yielded an oil-based extract exhibiting a dark brown and greenish hue.

2.3. QUALITATIVE PHYTOCHEMICAL ANALYSIS:

The qualitative phytochemical study was performed on the extracts by using below standard tests *(Nirosha et al., 2019).*

2.3.1 Test for Alkaloids (Wagner's test)

About few ml of plant extract was treated with 4-5 drops of Wagner's reagent. The formationof reddish- brown precipitate confirms the presence of Alkaloids

2.3.2 Test for Phenol (Ferric chloride test)

About 2ml of the extract was treated with 10% ferric chloride solution and observed for the formation of deep blue / black colour.

2.3.3 Test for Saponins (Foam test)

To 2 ml of the plant extract added 6ml of water in a test tube. The mixture was shaken vigorously and observed for the formation of persistent foam for few seconds. The presence offoam confirms the presence of saponins.

2.3.4 Test for Flavonoids

To about 2ml of plant extract, few drops of 10% ferric chloride solution was added. The formation of green or blue colour indicates the presence of flavonoids.

2.3.5 Test for Tannin

To about 1ml of plant extract added few drops of dilute ferric chloride solution. The presenceof tannin is confirmed by the formation of dark green or blue color.

2.4 Quantitative of Phenols, Flavonoids, Tannins and Saponins (Shahraki et al., 2023)

2.4.1 Estimation of Phenols

The total phenolics content in different solvent extracts was determined with the Folin- Ciocalteu's reagent (FCR). In the procedure, different concentrations of the extracts were mixed with 0.4 ml FCR (diluted 1:10 v/v). After 5 min 4 ml of sodium carbonate solution wasadded. The final volume of the tubes was made up to 10 ml with distilled water and allowed to stand for 90 min at room temperature. Absorbance of sample was measured against the blank at 750 nm using a spectrophotometer *(Fattahi et al., 2014).*

2.4.2 Estimation of Flavoniods

To 1ml of test extract 5 ml pH 4.7 phosphate Buffer was added and 5 ml BCG solution and shake a mixture with 4 ml of chloroform. The extracts were collected in a 10-ml volumetric flask and then diluted to adjust volume with chloroform. The absorbance of the complex in chloroform was measured at 470 nm against blank prepared as above but without extract. Atropine is used as a standard material and compared the assay with Atropine equivalents*(Ajanal et al., 2012).*

2.4.3 Estimation of tannin

The 1 ml of the sample extracts of concentration 1mg/ml was taken in a test tube. The volumewas made up to 1ml with distilled water and 1 ml of water serves as the blank. To this 0.5 mlof Folin's phenol reagent (1:2) followed by 5ml of 35% sodium carbonate was added and kept at room temperature for 5 min blue colour was formed and the colour intensity was read at 640 nm*(Pokhriyal et al., 2023).*

2.4.4 Estimation of saponin

The Aluminium chloride method using catechin as a standard. 1ml of test sample and 4 ml ofwater were added to a volumetric flask (10 ml volume). After 5 min 0.3 ml of 5 % Sodium nitrite, 0.3 ml of 10% Aluminium chloride was added. After 6 min incubation at room temperature, 2 ml of 1 M Sodium hydroxide was added to the reaction mixture. Immediatelythe final volume was made up to 10 ml with distilled water. It measured at 510 nm against a blank spectrophotometrically(*Akullo et al., 2023*).

Test extract was dissolved in 80% methanol, 2ml of Vanilin in ethanol was added, mixed welland the 2ml of 72% sulphuric acid solution was added, mixed well and heated on a water bathat for 10min, absorbance was measured at 544nm against reagent blank(V. *Le* et al., 2018).

2.5 GC-MS analysis:

GC-MS analysis was performed using SHIMADZU GC-MS QP 2010 using CARBOWAX capillary column and Helium as carrier gas to quantify the major phytochemical. 0.2µl of extract was injected in to the column at the flow rate of 1µl/minute. The injector was operatedat 250^{o} C and the oven temperature was programmed as follows; 60^{o} C for 15minutes, then gradually increased to 280^{o} C at 3 minutes. The identification of components was based on comparison of their mass spectra with those of Wiley and NBS libraries and those described by Adams as well as comparison of their retention indices *(Jassal & Kaushal, 2019; Manjamalai& Grace, 2012)*

2.6 DPPH radical scavenging activity

Various concentrations of the oil (1.25 µl, 2.5 µl, 10 µl, 25 µl, 80 µl ,100 µl, 200µl), derivedfrom a ratio of 1:1 oil in methanol was added to 2.5 ml of 0.004% DPPH solution. Then madethe volume to 3 ml by adding methanol. The mixture was allowed to react at room temperaturefor 15 to 20 minutes. Methanol served as the blank and a tube without the extracts served as the positive control. After 30 minutes of incubation, the discoloration of the purple colour wasmeasured at 517nm in a spectrophotometer. (S. et al., 2016).Sample blank was prepared for coloured sample. The assay was calculated as:

3. Results

3.1 Yield Calculation and Qualitative analysis for GSAE and GSO

GSO and GSAE were extracted using hydro distillation and Soxhlet methods, respectively, resulting in different outcomes for each sample. Yield calculations, as well as qualitative and quantitative studies, were performed. GSAE, with a higher yield of 2.7%, proved to be a goodsource of alkaloids and tannins, and contained a significant amount of saponins. In contrast, GSO had a yield of 1%, with a higher presence of alkaloids and tannins but comparatively lesssaponin.

Percentage of yield (%) = Total of quantity samples/ Total of quantity yield*100

Table 1: Comparative yield analysis of GSAE and GSO

S.no	GSAE	GSO
1	**Physical appearance:** The extract was **brown in colour in powder form**	**Physical appearance:** The oil colour is **greenish-brown colour**
2	Total yield of the **GSAE was 2.7%**	Total yield of the **GSO was 1%**

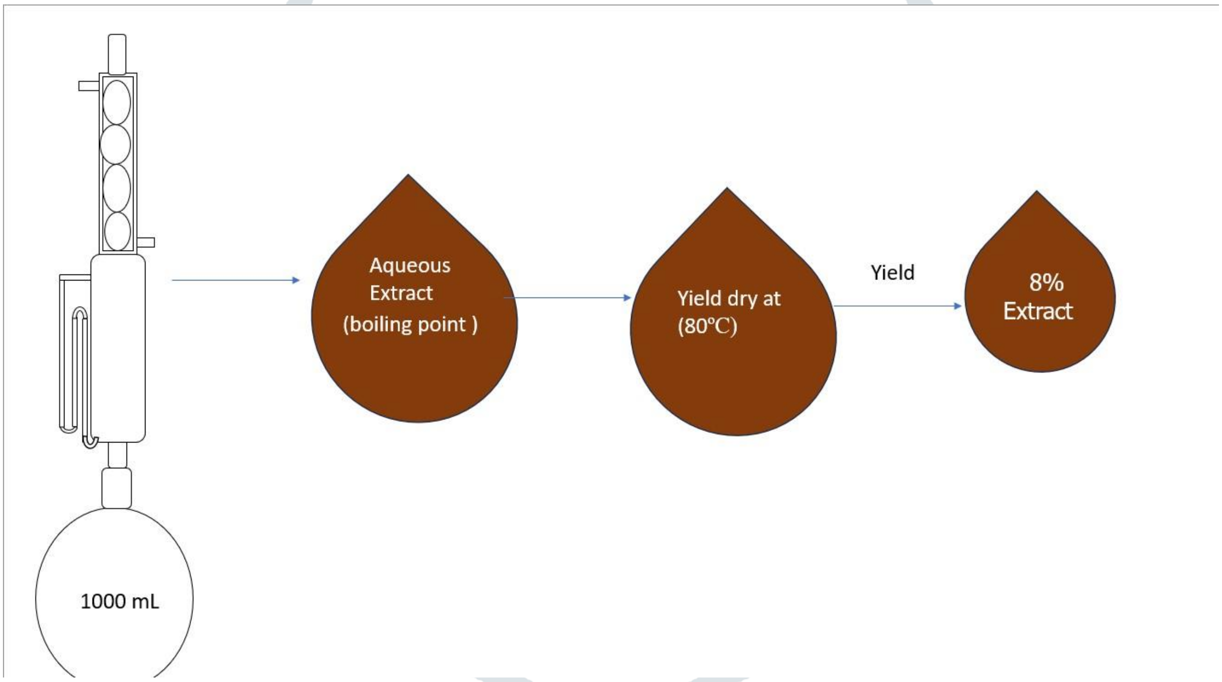


Figure 2: Method and yield for the GSAE

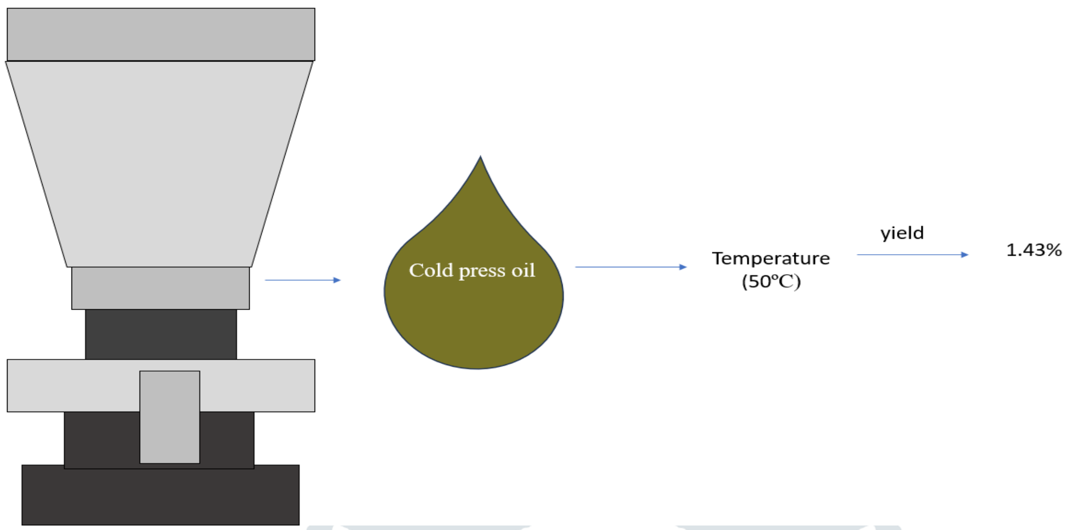


Figure 3: Method of extraction and yield for the GSO

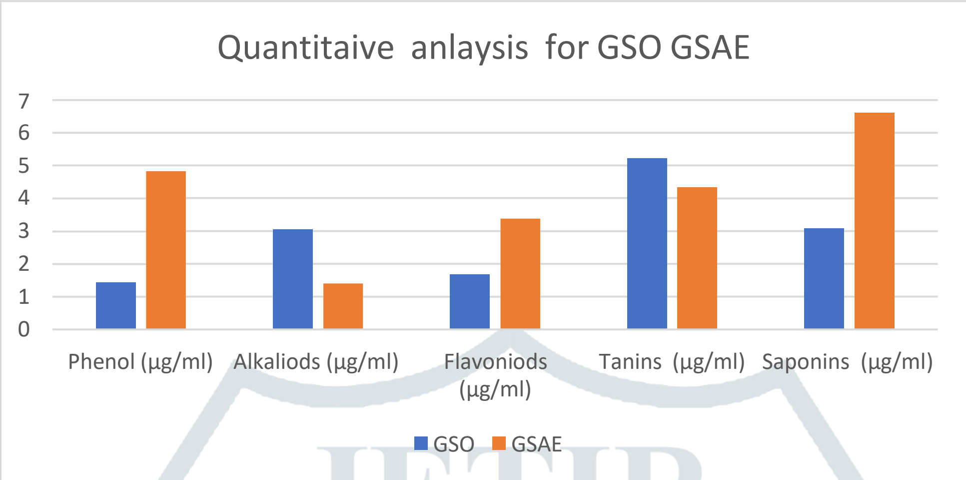
Table 2: Qualitative Analysis of GSAE and GSO

S.NO	TEST	OBSERVATION	GSAE	GSO
1	Alkaloids	**Reddish brown precipitate**	++	+++
2	Flavonoids	**Yellow colour**	+ +	+
3	Phenol	**brown colour**	+ ++	+ +
4	Protein	**Purple or violet colour**	+	+ +
6	Tannins	**Dark green or blue colour**	+ +	+++

Table 3: Estimation Of Phenol, Alkaliods Flavonoids, Tannins And Saponins

S.no	Name of samples	Phenol (µg/ml)	Alkaliods (µg/ml)	Flavoniods (µg/ml)	Tanins (µg/ml)	Saponins (µg/ml)
1	GSO	1.42656	3.056911	1.660886	5.22	3.085714
2	GSAE	4.826	1.397239	3.3765	4.3332	6.613048

Graph 1: Quantitaive anlaysis for GSO and GSAE



3.2 GC-MS results for GSO and GSAE

The chromatogram obtained through GC-MS analysis of GSO exhibited the presence of40 compounds. Relative concentration, as indicated by peak area percentage, allowed for the assessment of each compound's abundance. Among these, Phytosterols were the most abundantconstituent, accounting for 8.56% of the total composition, followed by Tocopherols at 0.12% and Triterpenoids at 1.18%. The significant presence of Phytosterols and Tocopherols suggeststheir potential as strong antioxidants. In contrast, the chromatogram of GSAE revealed the presence of 60 compounds. Relative concentrations were determined through peak areapercentages, highlighting Isoscutellarein and Scopoletin as the major compounds, constituting 12.29% of the total composition. These findings emphasize the antioxidant potency of Isoscutellarein and Scopoletin, indicating their substantial contribution to the extract's antioxidant activity. Overall, the analysis underscores the diverse chemical composition of both GSO and GSAE, with particular attention to the antioxidant potential associated with the identified compounds, thus providing valuable insights into their potential applications in various fields.

Figure 4: GC-MS for GSAE

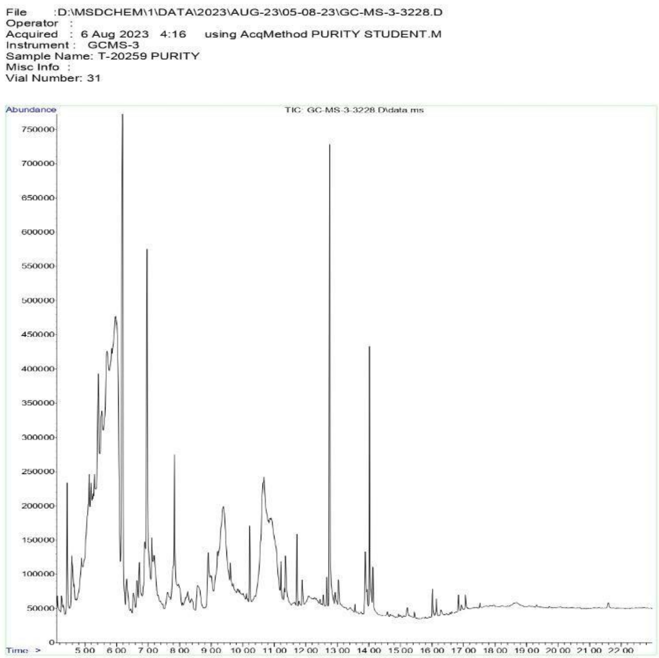


Table 4: Major secondary metabolites present in GSAE According GC –MS result

RT time	Area %	Compound	Common name	Classes	Therapeutic uses
6.187	12.29	4H-Pyran-4-one,2,3 dihydro-3,5	Isoscutellarin	Flavoniod	Anti-oxidant
13.03	0.64	9H-Pyrido[3,4-b]indole, 1 methyl-	Skatole	Alkaliod	
16.93	0.14	Z-6-Pentadecen-1-ol acetate	Z-6-Pentadecen-1-ol acetate	Terpeniods	Anti-inflammatory

Figure 5 : GC-MS for GSO

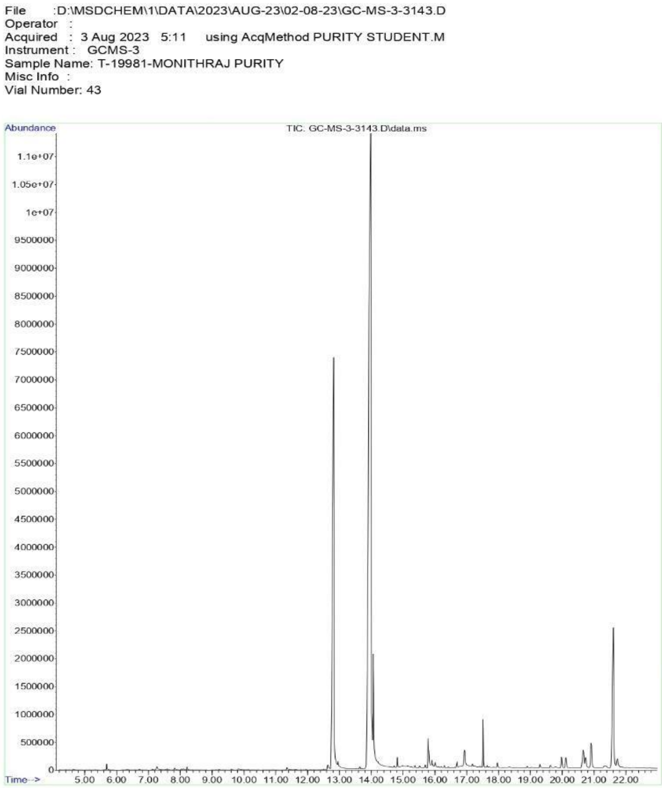


Table 5: Major secondary metabolites present in GSO According GC –MS result

RT Time	Area%	Compound	Common Name	Classes	Therapeutic use
17.507	1.18	Squalene	Squalene	Terpeniods	Anti-oxidant
19.62	0.12	Vitamin E	Tocopherol	Tocopherol	Anti- Oxidant
21.607	7.43	Ergost-en-3-ol	Phytosterol	Phytosterol	Anti- inflammatory
20.907	1.13	Stigmasterol	Phytosterol	Phytosterol	Anti –oxidant

3.3 Anti-oxidant activity

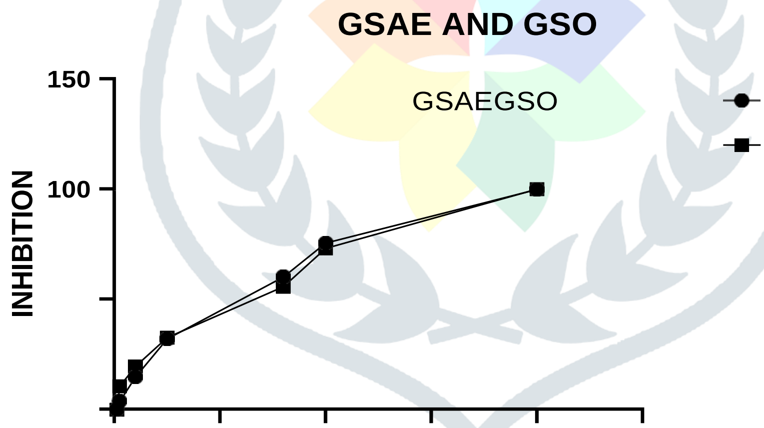
The DPPH free radical scavenging assay was performed using a varied concentration range of 1.25–200 µg/mL and the IC_{50} values for both GSAE and GSO was established. Notably, it was observed that GSO exhibited the strongest antioxidant activity among all samples.as IC_{50} lowest compare to GSAE . And the data shown in Table 6 and Graph

2.

Table 6 : Percentage of the Inhibition for GSAE and GSO

Concentration	Inhibition percentage of GSAE	Inhibition percentage of GSO
1.25	0.9448	9.508
2.5	8.3464	12.836
10	14.645	22.820
25	23.779	38.193
80	39.842	63.391
100	51.968	77.179
200	70.708	99.049
IC_{50} (µg/mL)	**98.170**	**72.38**

Graph 2: Percentage of the Inhibition for GSAE and GSO



50

0

0 50 100 150 200 250

Concentration

4. Discussion

It is one of commercial and an ancient royal fruit in the roman civilisation(Ma & Zhang, 2017). The weight of an induvial berry is 0.050 g in which one-by third of the weight is covered by the seed (0.02g) (Elejalde et al., 2022) . The seed present in some of the grape variety .It contains rich sourcesof secondary metabolites compounds that can be categorized into volatile (fatty acids and secondarycompounds) and non-volatile (fragrance) compounds (Yalcin et al., 2017). It has been less quantity of non-volatile compounds present in it. The volatile compounds are stored in the form of 8% oil and92% flesh form.

The Compounds have the unique physical (ph, temperature and appearance) and chemical (structureand function) properties .The volatile compounds was categories on their temperature bases that present Grape seed .The ketones (56ºc), esters(77ºc), aldehydes (vapour at room 30-37 ºc) and alcohols (77ºc) are the volatile compounds present in the seed. The ester , aldehydes , ketones low percent and absent of the alcohol in the GSO. The GSAE also contains low percent of the volatile compounds due to thermo-effect.

Through which the secondary metabolites were obtained which were present in the solid material. The GSAE was extracted at the boiling point (above 100ºc) and dried at (80 ºc).The GSO was extracted at a moderate temperature (50 ºc) and preserved in room temperature (37ºc).The yield andthe quantity of the compounds have been calculated for both GSAE and GSO. In that, the GSAE is greater than GSO. The phytochemical screening (alkaloids, terpenoids, terpenes, phenol, flavonoids, saponin and tannins) and biological assay (GC-MS and antioxidants activity) were carried out for theGSO and GSAE. In the Phytochemical screening two types of studies were conducted: Qualitative and Quantitative analysis . The qualitative and quantitative studies showed that GSAE is a good source of flavonoids and GSO is a good source of alkaloids and tannins. More percent of flavonoids(12%) was present in the GSAE and absent of the phytosterol due to the chemical structure and solubility characterics. The flavonoid (isoscultellarion) temperature resistance compounds have the anti-oxidant properties. and higher quantity of phytosterols (8%) were present in the GSO. Gentle the flavonoid (isoscultellarion) compounds absent in the cold press (mechanical pressing) method behalf of the nature properties. The phytosterol (stigmasterol) compounds present in oil derived fromthe cholesterol form the fatty acids and tocopherol (vitamin E) have high potency anti-oxidant properties denature at 60ºc.The anti-oxidant activity was obtained by the DPPH assay. In this assay the absorbance of the charged electron was neutralized in low contraction.

According to the hypothesis , the extract has a huge quantity of the compounds that has a promisingresult for the studies. But sometimes it might differ from the natural theory. The oil extract has the lower quantity of yield and compounds compared to other extracts. The previous studies mentioned that GSAE has the Strong IC_{50} concentration values 88 µg/ml and lower IC_{50} concentration mentioned (Leifert & Abeywardena, 2008). GSO the bioactive compounds that are present in the oil hasgood activity compared to the aqueous extract . It shows, that the antioxidant analysis using the DPPHscavenging assay. In this study, the GSAE have IC_{50} concentration values of 93.170 µg/ml and the GSO have IC_{50} concentration values of 72.38 µg/ml. The results shows that both methods of extraction have a strong antioxidant activity. According to overall results GSO has the good inhibitory concentration values compared to GSAE.

5. Conclusion

In conclusion, our study unequivocally demonstrates that the oil derived from *Vitis vinifera* .L (Grapeseed) exhibits noteworthy and robust antioxidant activity in scavenging free radicals, surpassing thatof the GSAE. This highlights the promising potential of GSO as a natural alternative to conventionalchemical drugs, offering therapeutic benefits without associated side effects .In further the GSO usedfor the *invitro and invivo* cancer studies.

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