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Determination of Reciprocal Potential of Ginger, Ashwagandha, and Milk Thistle Using Various Assays

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KEYWORDS

Ayurveda plants, Plant extracts, Phytoconstituents, Antioxidant, Anticancer, Toxicity

ABSTRACT:

From the ancient period, ayurvedic treatment has played a vital role in medical development. Despite great technical breakthroughs, cancer remains the top cause of death worldwide. The study has highlighted various perceptions of plant extraction and its characteristics including the different plant parameters. Collecting plants and executing Soxhlet technique, qualitative analysis of phytochemicals has been done with the plant's sample which has been briefly interpreted. The quantitative phytochemical analysis of phenols, flavonoids, tannins in extract was estimated using standard curve of Gallic acid, Quercetin and Tannic acid respectively. In this particular study, Ginger, Ashwagandha, and Milk Thistle are focused to evaluate their impact on human well-being. Synergism and its influential benefits on the plants' characteristics have been analysed. Antioxidant parameters of plants activity have been used in this study to compare the effectiveness of the plant extractions on the basis of their concentration ability. The antioxidant potentiality of plants also has been analysed in this study taking into account their different forms such as raw or prepared or combined characteristics. FTIR analysis and Toxicity tests also have been conducted to evaluate the potential of plant extraction. The graphical interpretation of the applied methods on plants has derived the result that highlighted the different effective characteristics of the plants such as Ginger and its characteristic helps in folk medicine, Ashwagandha can be considered an effective application in nerve diseases.

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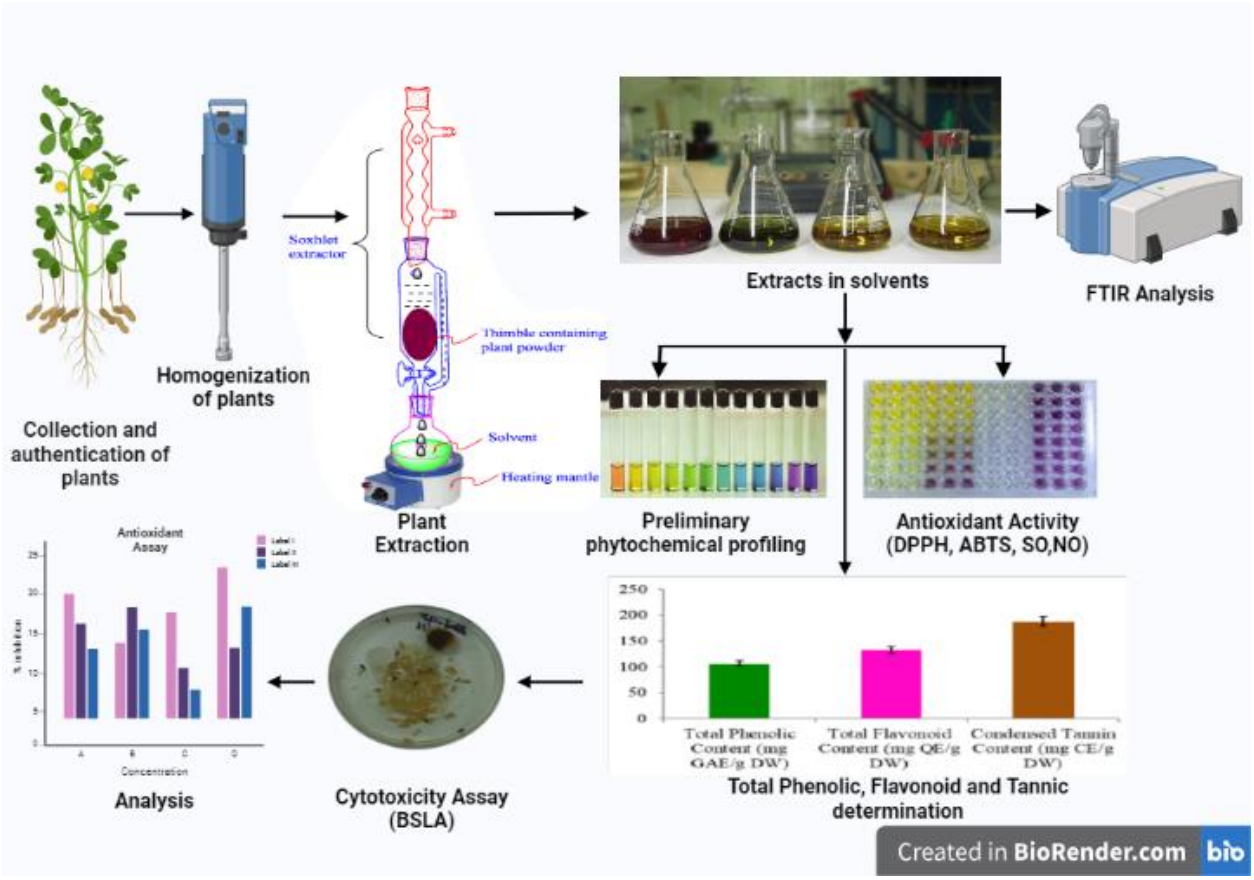

1. INTRODUCTION

Our planet, which is commonly referred to as a "green planet" because of the prevalence of plants, is shielded by them. On the other hand, due to their unparalleled chemical variety, plants provide an almost infinite number of opportunities to find new drugs (Cos *et al*., 2006). From generation to generation, traditional medical knowledge is transmitted verbally, thus it is possible that fundamental details like the sections of plants used, the processes used to make drugs, the ailments treated, and so on, may be forgotten or lost in the process. So, it is essential to record the information about these medicinal plants in order to identify potential species for the creation of therapeutic drugs (Tibebu Alemu *et al*., 2018).

A family of plants that include *Zingiber officinale* (ginger). The main source of ginger's strong smell is its gingerols, which appear to be the main ingredient investigated in the majority of studies pertaining to health. Ginger has been used for thousands of years to treat everything from colds to cancer, and it is thought to have many powerful medicinal and preventative effects. Many disorders are linked to oxidative stress, and ginger's antioxidant capabilities are a typical mechanism usually suggested to explain its effects and health advantages (Aeschbach *et al*., 1994; Ahmad *et al*., 2001). Mallikarjuna et al. (2008) state that ginger has been shown to mitigate ethanol-induced hepatotoxicity by protecting against age-related oxidative stress markers and by reducing oxidation effects in rats fed ethanol. Numerous cancer types have been studied for ginger's potential to prevent or reduce the spread of these cancers.

Withania somnifera is also known by the names ashwagandha and winter cherry. In India, ashwagandha is a commonly used home treatment since it is considered the finest tonic for elderly people and children (Kulkarni et al., 2008). According to Ayurveda, the oldest medical system, it is one of the most potent nervine tonics. Clinical evidence has shown that continuous Ashwagandha administration improves neurological conditions such as brain strokes that cause paralysis and

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neuronal insufficiency (Syed et al., 2021). Additionally, it is utilised to treat all cancer kinds, including lung and prostate tumours, particularly in their advanced stages, offering patients a host of health benefits (dushani L *et al.*, 2015).

Silymarin, a significant component, is mostly found in the plant & leaves, roots, seeds, and fruit [Napolitano *et al*.,2013]. Nevertheless, Silymarin, which is likewise implicated in the regeneration of deteriorated hepatic tissues, is found in comparatively higher concentrations in the ripped seeds of *S. marianum* (Jayaraj *et al*., 2007). A key component of silymarin, silybin, decreases oxidative stress, lowers insulin resistance, and reduces fat storage in the human liver (Federico *et al.,* 2017). The primary elements of natural plant extracts are secondary metabolites, which exist as phenols and flavonoids and function as antioxidants (Kasote *et al.*, 2015). The considerable antioxidant capabilities of *S. marianum* make it an essential plant as well (Li *et al*., 2011). Furthermore, natural antioxidants are increasingly gaining attention in cutting-edge scientific research (Sanchez-Moreno *et al.,* 1999). These plant-based natural antioxidants have anti-viral, and anti-ageing characteristics (Cook *et al*., 1996).

Synergism is an effective process that helps to derive the combined potential of two components or substances which is more effective rather than their individual application (Angane *et al*. 2022). The combined effect of Ashwagandha and Silymarin can be more effective despite its individual impact. Ashwagandha extract is useful for nerve diseases whereas Silymarin is much more effective in reducing fat in the liver. Therefore, the synergism application of these two plants can be more beneficial for complex diseases considering their components' unique characteristics. Similarly, the combination of raw ginger and prepared ginger has more significant antioxidants that help to enhance the immune resistance of a human.

In the present study, The extract was prepared using the soxhlet extraction method.Antioxidant assay like DPPH, superoxide assay, nitric oxide assay, ABTS assay was performed by using *Zingiber officinale* roots, Milk Thistle seeds, and *Withaniasomnifera* leaves and were extracted and characterized using several qualitative and quantitative assays.

2. MATERIALS AND METHODS

2.1 Collection of Plant Sample:

The *Zingiber officinale* root sample was collected from the local market. The *Withania somnifera* leaves were collected from Zydus Botanical Garden, Ahmedabad. The *Silybum marianum* seeds were purchased online from INDIANJADIBOOTI.

2.2 Authentication of Plant sample:

All the three-plant samples were authenticated by Dr. Hitesh Solanki. Professor, Department of Botany, Gujarat University.

2.3 Extraction of secondary metabolites using the soxhlation method:

The roots of *Zingiber officinale*, leaves of *Withania somnifera*, and seeds of *Silybum marianum* were washed thoroughly, shade dried at room temperature, and grinded into fine powder form. There are 2 samples of *Zingiber officinale*, one is raw ginger powder and the other one was industrial-prepared ginger powder. Then the root, leaves, and seeds powder were passed through methanol and distilled water (70:30) simultaneously. The extractions were carried out at 65˚C for 24 hours.

$$Percentage\ yeild\ = \frac{weight\ of\ the\ extract\ after\ solvent\ evap}{weight\ of\ the\ powder\ used\ in\ Soxhlet\ sy}$$

2.4 Qualitative analysis of phytochemical:

Qualitative analysis is generally performed to check the presence or absence of photochemical constituents. For each metabolite, there is a specific test. Various test was carried out to confirm the presence of secondary metabolites.

2.5 Quantitative analysis of phytochemicals:

2.5.1 Determination of TPC:

With a few minor adjustments, the Folin-Ciocalteu colorimetric method as reported by S. Ojha et al., 2018, was used to calculate the TPC of the samples. The total phenolic content was determined using the Folin-Ciocalteu method. 1 ml of clear water, 1 milligramme of extract (10 mg/10 ml), and standard gallic acid (10, 20, 30, 40, and 50 µg/ml) were added. After adding, combining, and shaking 500 µl of the sample and 500 µl of Folin-Ciocalteu's reagent (2N) were used. After adding 1 millilitre of 1% sodium carbonate, the mixture

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was allowed to sit at room temperature for 45 minutes. The colour deep blue was produced. Using spectrophotometry, absorbance at 760 nm was measured following incubation. A solvent and reagent blank were used for the blank test.

2.5.2 Determination of TFC:

The aluminium chloride colorimetric method was used to calculate the TFC (Ojha et al., 2018). One milligramme of extract was combined with one millilitre of distilled water (10 mg/10 ml) in test tubes to create standard quercetin (10, 20, 30, 40, and 500 g/ml). Next, withdraw 250µl of the specimen. Next, pour distilled water into a 2.5 ml capacity and stir well. The hue that was achieved was orange-yellow. The absorbance was measured spectrophotometrically at 510 nm. For the blank test, a solvent and reagent blank were utilised. Using standard quercetin, the calibration curve was produced. The amount of total flavonoids per 100g dry mass was expressed as mg of quercetin equivalents.

2.5.3 Determination of TTC:

According to S. Ojha et al. (2018), TTC was computed using their method. After progressively combining the extracts (500 µl; 1 mg/ml stock solution), distilled water (8 ml), 0.5 ml of 0.1M FeCl3, and 0.5 ml of 8 mM potassium ferricyanide, the mixture was let to settle at room temperature (272°C) for approximately 10 minutes. I used a spectrophotometer to measure the absorbance at 720 nanometers. Adequate reagent blanks were prepared for every solvent, without the addition of sample.

2.6 Assessment of antioxidant activity:

2.6.1 DPPH scavenging activity:

The DPPH was removed following the method outlined by (Emad A. Shalaby et al., 2012). Make a 0.1mM DPPH-containing methanol solution. Make extract concentrations of 10, 20, 30, 40, and 50 µg/ml. As a standard, make various amounts of ascorbic acid. Each test tube should contain one millilitre of sample and one millilitre of DPPH solution. Then, fill each test tube with 200 µl of Tris HCl.

2.6.2 ABTS:

The capacity of scavenging ABTS was evaluated as described by Emad A. Shalaby *et al.*, 2012. Create a stock solution with 2.4 mM potassium persulfate and 7 mM ABTS. Mix the two equally and let it sit in a dark place for 14 hours. Once the ABTS solution has been produced, measure the absorbance at 734 nm at 0.700 using 60 cc of methanol. One millilitre of freshly extracted plant material and one millilitre of prepared ABTS solution were allowed to react for seven minutes at room temperature before the absorbance at 734 nm was measured using a spectrophotometer. As a benchmark substance, ascorbic acid was used.

2.6.3 NO antioxidant assay:

The method was chosen to examine the ability of plant extracts to scavenge nitric oxide radicals. Make extract concentrations of 10, 20, 30, 40, and 50 µg/ml. As a standard, make various amounts of ascorbic acid. Each test tube should have 250l of sample, 250l of PBS, and 1 ml of 10mM Sodium Nitroprusside. After 150 minutes at 25o C, add 500l of Griess reagent. Samples and the blank's absorbance levels are measured at 540 nm. Subtracting the absorbance of the blank from the sample is the next step. The findings are displayed as the standard substance's equivalent or as a percentage of inhibition.

2.6.4 Superoxide radical scavenging assay:

A 3 ml reaction mixture included 0.3 ml of different concentrations (10–50 µg/ml) of sample solution, 0.75 ml NADH (936 µM), 0.75 ml NBT (300 µM), 0.75 ml PMS (120 µM), and phosphate buffer (100 mM, pH 7.4). After 5 minutes of incubation at room temperature, the amount of formazan generated was measured by comparing the absorbance at 560 nm to an appropriate blank. Ascorbic acid was the positive control used.

2.7 FTIR Analysis:

FTIR-ATR spectroscopy was used to analyze a plant extract to identify functional groups (Bruker, Germany). A 2 ml sample was obtained for examination, and it was filtered using a Poly-ether sulfone membrane-equipped 0.2-micron syringe filter from Millipore Inc. For 15 minutes, filtered samples were subjected to sonication to remove any remaining dissolved oxygen bubbles. The sample was then dropped into a diamond stage. Results were analyzed using OPUS spectroscopy software. The results were described in detected wavelength and functional groups for each sample.

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2.8 Toxicity test using the BSLT method (Brine Shrimp Lethality Test):

Concentrations of 10, 20, 30, 40, and 50 µg/ml were calculated to make the samples more concentrated. In accordance with the concentration calculation, 1 mL of the test sample was obtained, placed in a test tube, and 1 mL of saltwater was added. There are two tubes in each concentration. After adding 10 nauplii and 200 µl of saltwater, the test sample was mixed. The number of dead and live larvae was counted after a 24-hour incubation period, and probit analysis was used to calculate the LC50 and LT50 values (Wu C., 2014)

3. RESULTS

3.1 Qualitative Examination of the Phytochemicals:

Qualitative analysis is generally performed to check the presence or absence of phytochemicals. There is a specialized test for each type of secondary metabolite.

Test	**Reagents**	**Observation**	**Results (*Zingiberofficinale* extract)**		**Results (*Withania somnifera* extract)**	**Results (*Silybum marianum* extract)**
			Raw powder	**Prepared powder**		
Carbohydrates	Molisch's test	Junction of two liquid	+++	+	++	+
	Fehling's test	Brick red colour	+	+	+	+
	Benedict test	Green- yellow colour	+	++	+	++
Flavonoids	Alkaline test	Yellow-orange colour	++	+	+	+
	Ammonia test	Yellow colour indicates	+	++	+	+
Proteins	Biuret test	Violet colour	+++	+	++	+
Amino acids	Xentho Proteins	Reddish orange color	+	+	++	+
	Folin test	Blue colour	+++	++	++	+
Steroids	Liberman's burchard's test	Reddish to green colour	++	+	+	+
	Salowski test	Red colour in the chloroform layer	+++	++	+++	++
Terpenoids test	Terpenoids test	Greenish colour appears	+	+	++	+++
	Liberman Burchard test	Reddish ring indicates	++	++	+	+
Quinones	Hydrochloride test	Yellow colour indicates	++	+	++	++
Caumarin	Sodium hydroxide test	Yellow colour	+	++	+	+

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Anthocyanin	Sulphuric acid test	Yellowish orange	++	+	+++	+
Glycoside	KellarKilliani test	Reddish brown/ blue green colour	+++	+	++	+
	Test for glycoside	Pink colour	+	++	+++	-
Oxalate	Glacial acetic acid test	Green colour	++	+	+++	-

Table 3.1.1 Results of Qualitative Assays

3.2 Quantitative screening of the phytochemicals:

Quantitative tests are performed to check the number of phytochemicals present in the extract. Since determining antioxidant activity was our goal, we also measured the total phenolic and total flavonoid contents, which may be related to antioxidant activity.

3.2.1 Determination of Total Phenolic Content:

- The gallic acid was taken as standard and its graph was plotted.

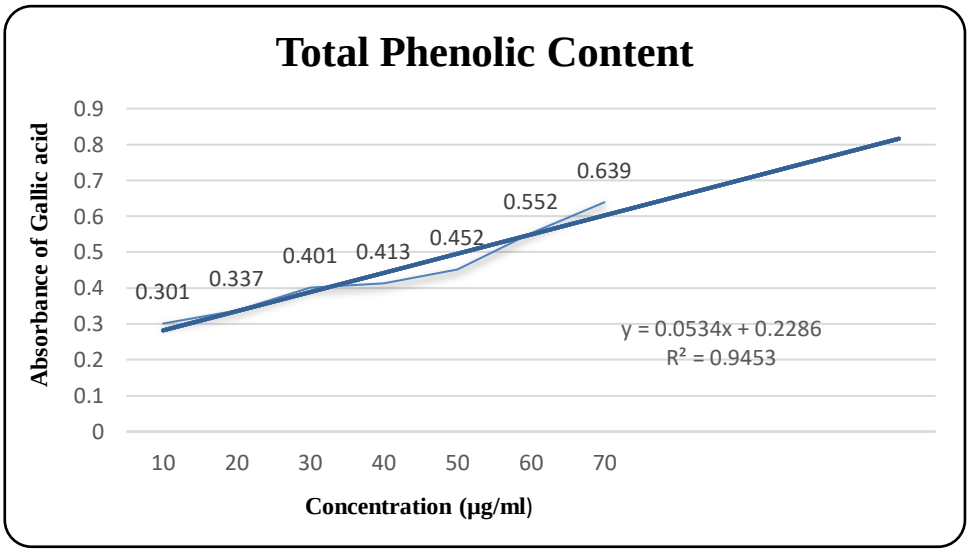
Calculation shows that TPC present in the extract of

1) Raw *Z. officinale* –144.6 mg GAE/g.

2) Prepared *Z. officinale* – 140.4 mg GAE/g.

3) *Withaniasomnifera* – 103 mg GAE/g.

4) *Silybum marianum* – 85.54 mg GAE/g.



Graph 3.2.1.1 Calibration curve for Gallic acid

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3.2.2. Determination of Total Flavonoid Content (TFC):

- The quercetin was taken as standard and its graph was plotted.

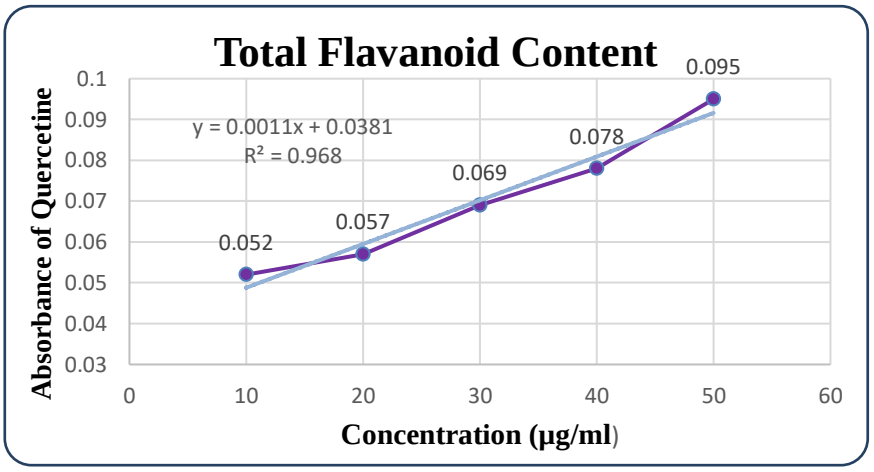
Calculation shows that TFC present in the extract of

1) Raw *Z. officinale* –798 mg QE/g.

2) Prepared *Z. officinale* – 598 mg QE /g.

3) *Withaniasomnifera* – 670 mg QE/g.

4) *Silybum marianum* – 616.36 mg QE/g.



Graph 3.2.2.1 Absorbance curve for Quercetin

3.2.3.1 Determination of Total Tannin Content:

- The Tannic acid was taken as standard and its graph was plotted.

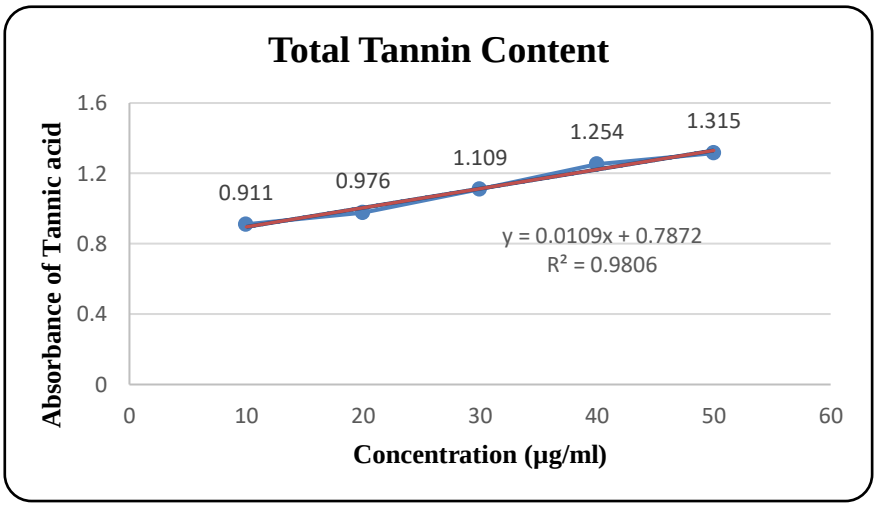
Calculation shows that TTC is present in the extract of

1) Raw *Z. officinale*–937.2mg TAE/g.

2) Prepared *Z. officinale* – 770.2 mg TAE/g.

3) *Withaniasomnifera* – 583.1 mg TAE/g.

4) *Silybum marianum* – 828.8mg TAE/g.



Graph 3.2.3.1 Calibration curve for Tannic acid

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Quantitative Screening of phytochemicals			
Extract	**Total Phenol**	**Total Flavonoid**	**Total Tannin**
Raw *Z. officinale*	144.6 ± 0.04 mg GAE/g	798 ± 0.007 mg QE/g	937.2 ± 0.07 TAE/g
Prepared *Z. officinale*	140.4 ± 0.04 mg GAE/g	598 ± 0.007 mg QE/g	770.2 ± 0.07 TAE/g
Withania somnifera	103 ± 0.04 mg GAE/g	670 ± 0.007 mg QE/g	583.1 ± 0.07 TAE/g
Silybum marianum	85.54± 0.04 mg GAE/g	616.36± 0.007 mg QE/g	828.8 ± 0.07 TAE/g

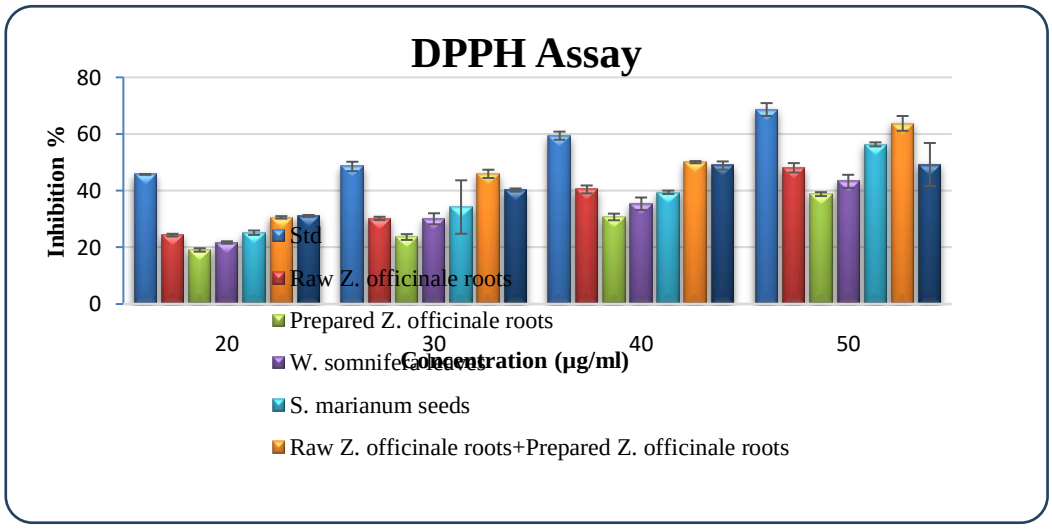
Table 3.2 Quantitative screening of phytochemicals

Values are performed in duplicates and represented as mean± SE

3.3 Assessment of antioxidant activity:

3.3.1. DPPH scavenging activity:

Colour change from purple to yellow was observed after incubation for 30 min in the dark which indicates antioxidant activity in the sample.



Graph 3.3.1.1 The graph shows the concentration of % inhibition between

Ascorbic acid and extracts (DPPH)

According to this graphical interpretation, raw ginger powder has an effective potential of antioxidants that increase the immune ability in the human body considering its inhibitory concentration. Raw ginger can be considered the most powerful immune booster whereas prepared ginger powder has low potential in antioxidant characteristics. Ashwagandha also can include in the antioxidant category.

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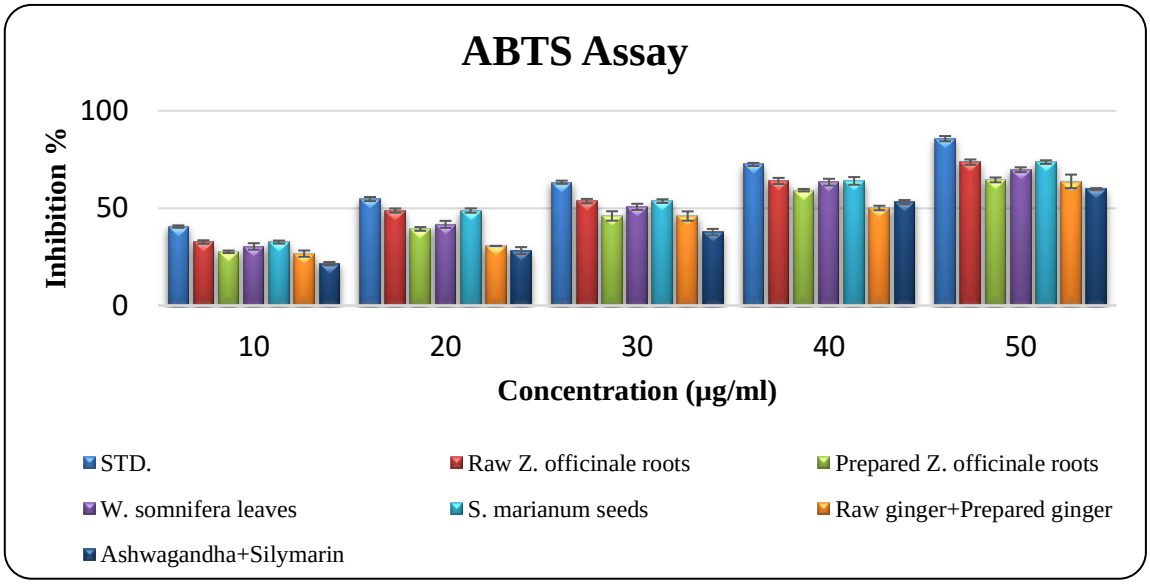
3.3.2. ABTS:

Colour change from Blue-green to pale-yellow was observed after incubation for 5min at Room temperature which indicates antioxidant activity in the sample.

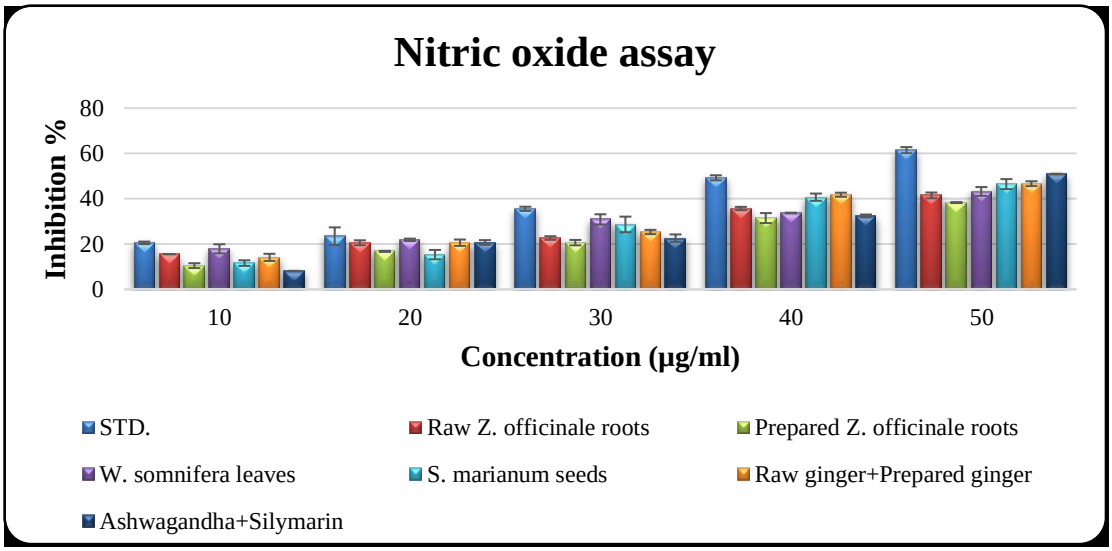


Graph 3.3.2.1 The graph shows the concentration of % inhibition between ABTS stock solution and extracts

ABTS, 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) observes the reaction kinetics of specific enzymes that helps in detecting the enzyme-based immunity ability of plant extracts. Raw ginger and Ashwagandha both have the capability of immunity boosters that helps in preventing diseases.

3.3.3. Nitric Oxide (NO) antioxidant assay:

Colour change from pale yellow to intense blue was observed after incubation for 150 min at Room temperature which indicates antioxidant activity in the sample.



Graph 3.3.3.1 The graph shows the concentration of % inhibition between Ascorbic acid and extracts (NO)

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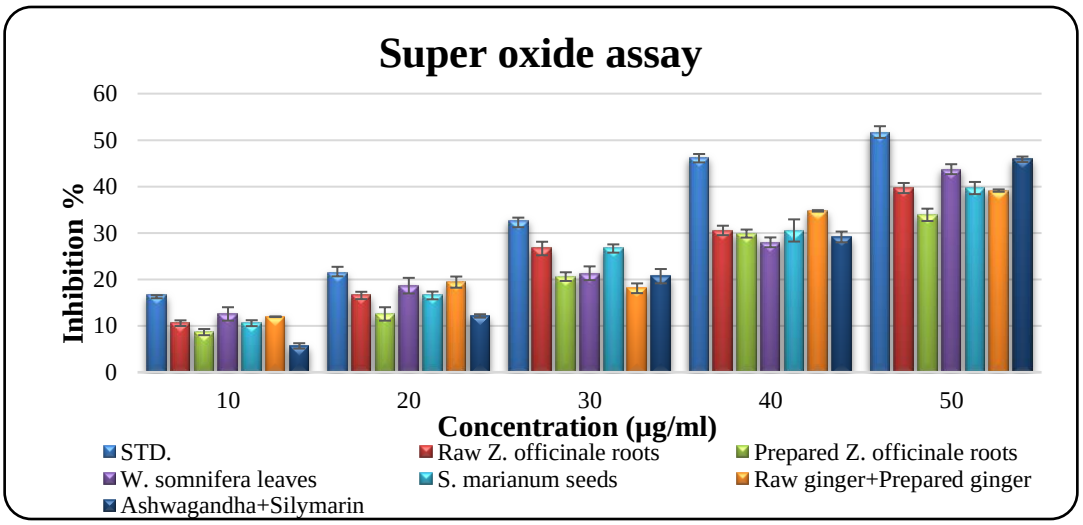
Nitric Oxide is one of the most effective chains-breaking antioxidants and the scavenging activity of the plant extracts against nitric oxide radicals can be evaluated through this procedure. The graph represents that Ashwagandha is one of the best sources of NO radical-based antioxidants that helps to prevent diseases.

3.3.4. Super Oxide (SO) antioxidant assays:

Colour change from colourless to red was observed after incubation for 30 min at Room temperature which indicates antioxidant activity in the sample.



Graph 3.3.4.1 The graph shows the concentration of % inhibition between Ascorbic acid and extracts (SO)

SO assay also highlighted in the graphical presentation that Ashwagandha has an effective impact on the immunity Increasement considering its high concentration of SO. The inhibit concentration power of Ashwagandha is more than 40% which is beneficial for the immune system.

3.3.5 Inhibition % at highest concentration values of plant extracts according to the assay.

Sample	DPPH	ABTS	SO	NO
ascorbic acid (std)	68.64	85.67	51.56	61.45
Raw Ginger	48.08	73.65	39.67	41.45
Prepared Ginger	38.78	64.51	33.9	38.3
Ashwagandha	43.29	69.73	43.73	43.21
Milk Thistle	56.35	73.65	39.67	46.45
Raw ginger + Prepared ginger	63.75	63.75	39.13	46.63
Ashwagandha + milk thistle	49.23	59.89	45.86	50.97

Table 3.3.5.1: Inhibition % at highest concentration

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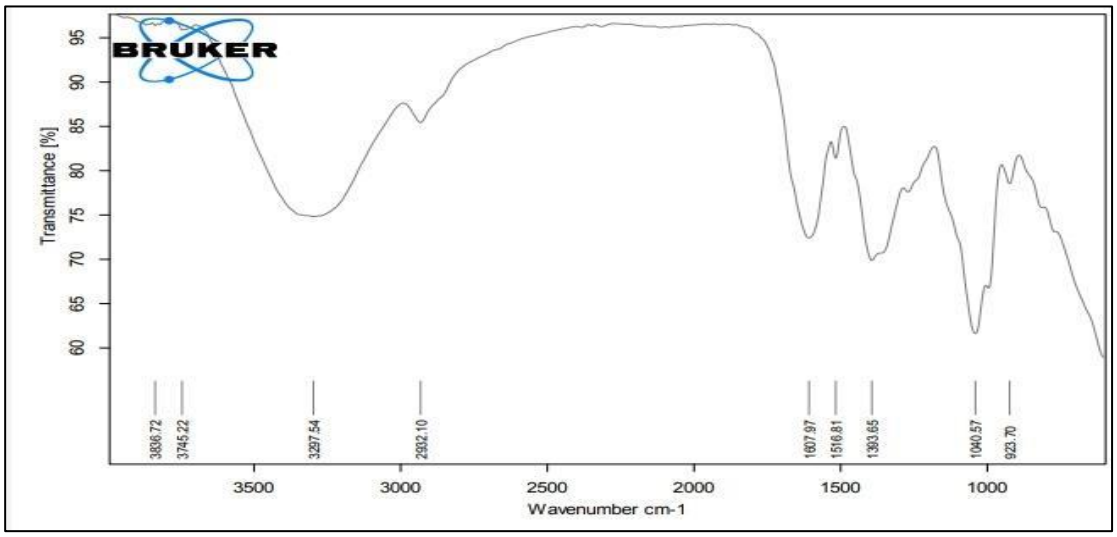
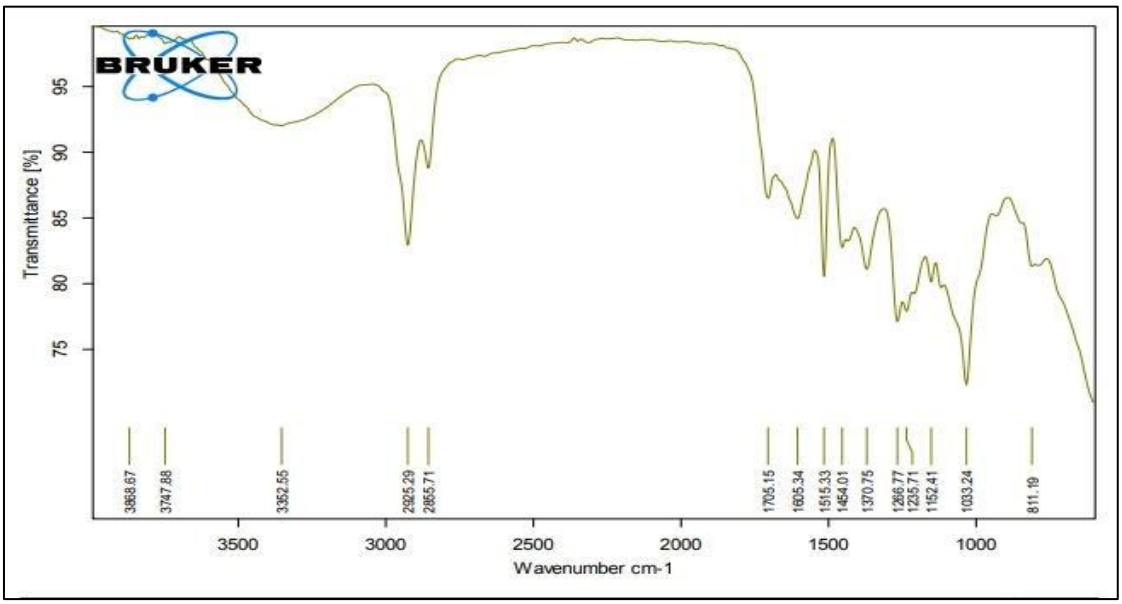
3.4 FTIR analysis:

The functional groups were analyzed using an FTIR spectrophotometer for extracts. The results obtained are presented in the figure given below which showed a diagrammatic representation the of FTIR spectrum. FTIR analysis showed the presence of various functional groups in extracts of *Z. officinale* and *W. somnifera*. The functional groups which we identified are in given below table.



***Zingiber officinale* (Raw Ginger Powder)**



Graph 3.4.2 The Graph represents the FTIR result of

***Zingiber officinale* (Prepared Ginger Powder)**

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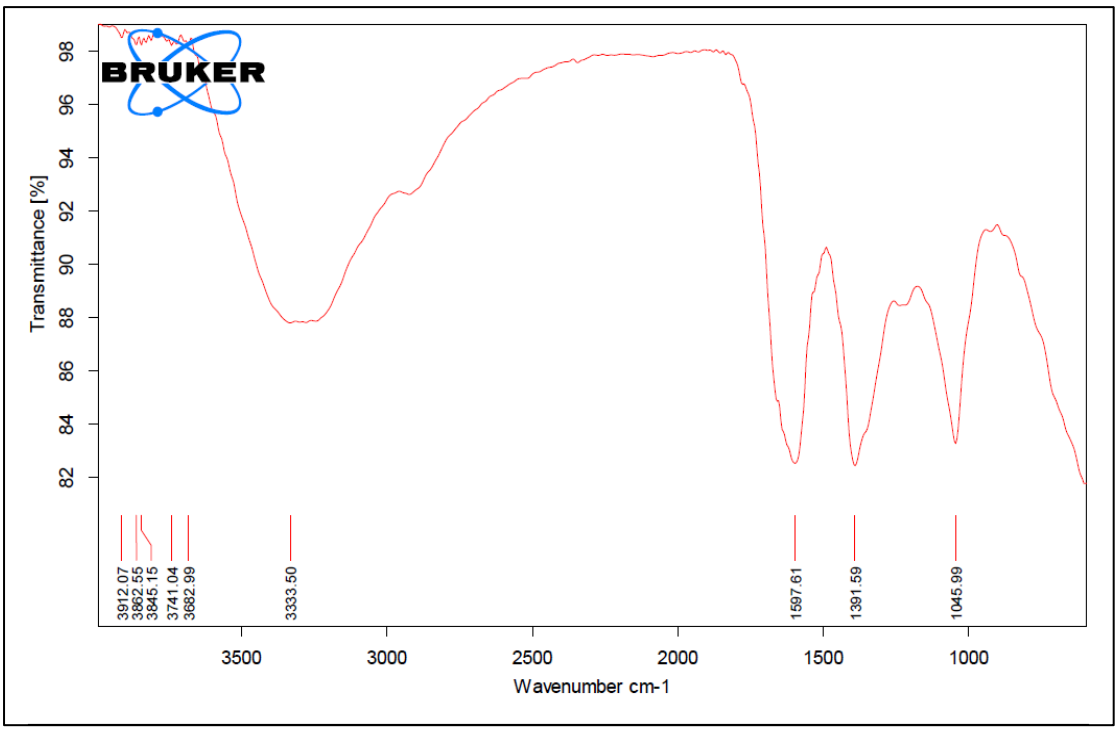
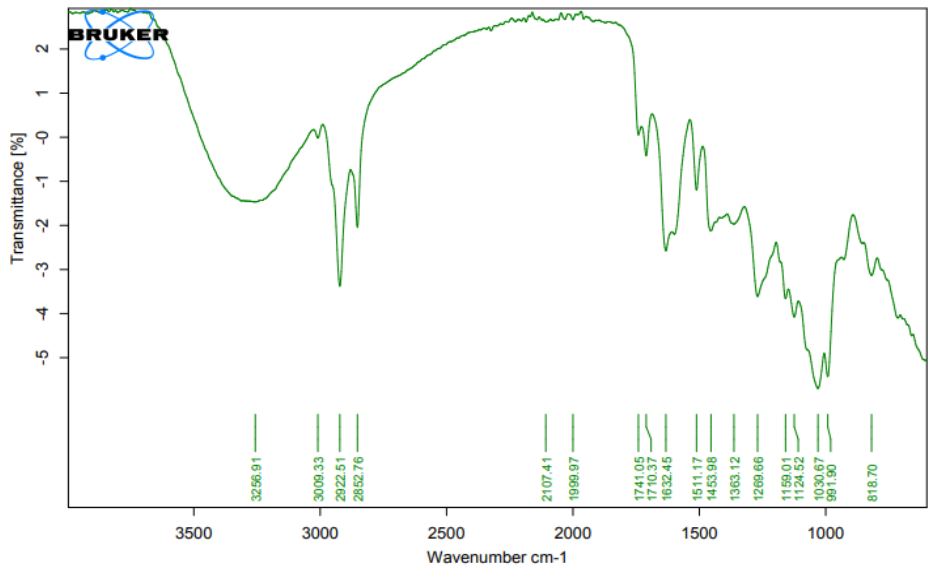


Graph 3.4.3 The Graph represents the FTIR result of

Withania somnifera



Graph 3.4.4 The Graph represents the FTIR result of

Silybum marianum

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Wavelength (Cm-1)	Functional group	Raw *Zingiber officinale* extract	Prepared *Zingiber officinale* extract	Withania somnifera extract	Silybum marianum extract
3550-3200 (O-H) 1420-1330 (O-H) 1085-1050 (O-H)	Alcohol	+	+	+	+
3000-2840 (C-H) 1450 (C-H)	Alkane	+	+	-	+
2830-2695 (C-H) 920-1050 (O-C-H)	Aldehydes	+	-	-	-
3400-3300 (N-H)	Aliphatic primary amine	-	+	-	+
1050-1040 (CO-O-CO)	Anhydride	-	+	-	-
1390-1310 (O-H)	Phenol	-	+	-	-
1342-1266 (C-N)	Aromatic amine	-	+	-	+
1650-1600 (C=C) 730-665 (C=C)	Alkene	+	+	-	+
1070-1030 (S-O)	Sulfoxide	-	-	+	+
1650-1566 (C=C) 1662-1626 (C=C)	Cyclic alkene	-	+	+	+
2260-2190 (C≡C)	Alkyne	-	-	-	-
2000-1900 (C=C=C)	Alene	-	-	-	+
1750-1735 (C=O)	Lactone esters	-	-	-	+

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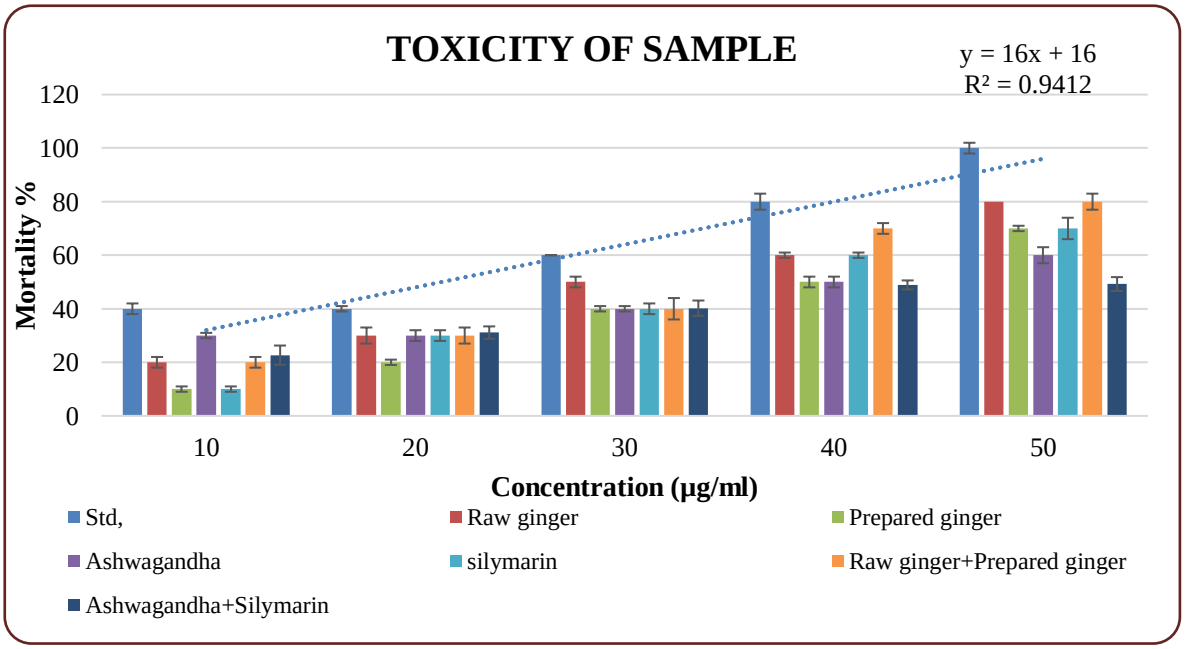
1710-1680 (C=O) **1710-1685 (C=O)**	**Conjugated acid** **Conjugated aldehyde**	-	-	-	+
2000-1650 (C-H)	**Aromatic compound**	-	+	-	+
1550-1500 (N-O)	**Nitro compound**	+	+	-	+
1395-1440 (O-H)	**Carboxylic group**	+	-	-	+
1400-1000 (C-F)	**Fluro compound**	+	+	+	+

Table 3.4.1 FTIR result of extracts showing Various functional groups

3.5 Toxicity test using the BSLT method (Brine Shrimp Lethality Test):

A magnifying glass is used to examine the vials after a 24-hour period, and the number of surviving nauplii in each vial is counted and noted. This information was used to determine the percentage of nauplii mortality for each sample concentration. The graph's straight-line equation was used to determine the samples' median lethal concentration (LC50). You can use the following formula to get the percentage of mortality:

$$\%\text{Mortality} = \frac{N_0 - N_1}{N_0} \times 100$$



Graph 3.5.1 Graph represents the mortality % of extracts and standard

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The assay's foundation was the extracts' or pure chemicals' capacity to destroy brine prawns developed in a lab, namely Artemia nauplii. The methodology described by Meyer et al. (Meyer, B.N., et al., 1982) and Lincoln et al. (Lincoln, R.D., et al., 1996) was followed when performing the experiment.

After 24 hours, the extracts were tested at various doses (10, 20, 30, 40, and 50 µg/mL) to determine their toxic impact on A. salina. As a reference standard, potassium dichromate was employed (Padmaja, R., et al., 2002). After 24 hours, the majority of plant extracts at a concentration of 100 µg/mL caused the complete (100%) death of brine shrimp nauplii.

Sample	**Mortality % at higher concentraion**	**LC_{50} value**
Std.(Potassiumdichromate)	100	21.25 µg/mL
Raw ginger extract	80	28.33 µg/mL
Prepared ginger extract	70	38 µg/mL
Withaniasomnifera extract	50	46.66 µg/mL
Silybum marianum extract	80	20.22 µg/mL
Raw ginger+Prepared ginger extract	70	36.75 µg/mL
Withaniasomnifera+ Silybum marianum extract	60	42.95 µg/mL

Table 3.5.1 LC_{50} values of extracts of BSLT

4. DISCUSSION

It is beneficial for several illnesses and primarily acts as a nervine tonic, which calms the nerves. Nowadays people have become more conscious about healthy life and the side effects of synthetic drugs. These plants are known to possess various activities like anti-inflammatory, anti-microbial, anti-ulcer, anti-cancer, anti-oxidant, etc. Thus, this study was focused to evaluate the phytochemistry of the plant and antioxidant activity of extract-prepared using soxhlation method.

The soxhlation method was carried out with two different solvents which include methanol and water. The existence of maximum secondary metabolites was detected from the extract, to confirm the presence of secondary metabolites qualitative estimation were performed. The phytochemical analysis and qualitative estimation of the extract of ginger, ashwagandha and milk thistle showed the presence of carbohydrates, phenols, terpenoids, flavonoids, quinones, glycosides, coumarins, and amino acids, and the same result was concluded by Baskaran, C., *et al*., 2012 found similar during his work on W. *somnifera* and Ahmed, N., *et al*., 2022 found similar during his work on Z. *officinale.* The determination of the reciprocal potential of natural substances like ginger, ashwagandha, and milk thistle using various assays is a significant area of research in the field of pharmacology and natural medicine. These three substances have been the focus of numerous studies due to their potential health benefits and medicinal properties. Let's delve into a discussion of this intriguing topic.

Ginger, scientifically known as Zingiber officinale, is renowned for its anti-inflammatory and antioxidant properties. Researchers have used various assays to assess its potential in combating oxidative stress and inflammation. These assays include DPPH (1,1-diphenyl-2-picrylhydrazyl) and FRAP (Ferric Reducing Antioxidant Power) tests to determine its radical-scavenging and reducing capabilities. These assays have

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revealed that ginger contains bioactive compounds capable of reducing oxidative damage in cells. Ashwagandha, or Withania somnifera, is an adaptogenic herb that has been studied extensively for its potential to reduce stress and anxiety. Researchers employ a range of assays, including cortisol level measurements, the Elevated Plus Maze test, and the Open Field test, to determine its effectiveness in alleviating stress-related conditions. The results suggest that ashwagandha may have a significant reciprocal potential in reducing stress and improving overall well-being. he determination of the reciprocal potential of natural compounds like ginger, ashwagandha, and milk thistle using various assays is a vital aspect of understanding their potential health benefits. These assays provide insights into the antioxidant, anti-inflammatory, and other bioactive properties of these herbs, which can be critical for their use in traditional medicine and as potential supplements in modern healthcare. Each herb possesses a unique set of bioactive compounds, and assessing their reciprocal potential can help in determining their suitability for various health applications.

Milk thistle, scientifically called Silybum marianum, is known for its hepatoprotective properties. Assays like the measurement of liver enzyme levels, histological analysis of liver tissue, and antioxidant enzyme assays are used to assess its potential in protecting the liver from damage. Studies have indicated that milk thistle may have a reciprocal potential in safeguarding the liver against toxins and oxidative stress. The determination of the reciprocal potential of ginger, ashwagandha, and milk thistle using various assays underscores their significance in the realm of natural medicine and pharmacology. These assessments provide valuable insights into the potential health benefits of these natural substances, paving the way for their use in traditional and modern healthcare practices. Further research is warranted to explore their applications and mechanisms of action in greater depth, potentially offering novel therapeutic solutions for various health issues.

Quantitative analysis in which the total levels of phenolic, flavonoid, and tannin were assessed using various assays. According to the assay, ashwagandha extract has a total phenolic content of (103 ± 0.04 mg GAE/g), (140.4 ± 0.04 mg GAE/g) for processed ginger powder, (144.6 ± 0.04 mg GAE/g) for Raw ginger powder, and (85.54± 0.04 mg GAE/g) for Milk thistle powder.Total flavonoid concentration revealed that ashwagandha contains (670 ± 0.007 mg QE/g) of total flavonoid content, prepared ginger powder contains(598 ± 0.007 mg QE/g), Raw ginger powder contains (798 ± 0.007 mg QE/g) and Milk thistle contains (616.36± 0.007 mg QE/g). Respectively, Raw ginger powder extract has a total tannin level of (937.2 ± 0.07 TAE/g), prepared ginger powder has (770.2 ± 0.07 TAE/g), Thereafter, ashwagandha extract has (583.1 ± 0.07 TAE/g) and Milk thistle extract has (828.8 ± 0.07 TAE/g) respectively. Thus, it can be stated that ginger, Milk thistle, and ashwagandha have higher levels of phenol, flavonoid, and tannin content, which is consistent with the findings of S. Ojha et al., 2018. Aside from the extraction process, many other factors influence the amount of phytoconstituents, such as ambient conditions, solvent type, temperature, and so on (Venkatesan et al., 2019). The higher the phenolic concentration, the more powerful the free radical scavenging effects. Researchers have also shown that plants high in phytochemicals such as phenols, flavonoids, and tannins are also high in antioxidants. (Pourmorad et al., 2006).

The slight variation found in this investigation was caused by the use of isolated organic solvents that exclusively removed specific components (Tiwari *et al*., 2006). The ability of various environmental factors, such as climate, rainfall, and other factors, to affect plant development and in turn, the quality and herbal compounds contained in particular species, even when they are grown in the same nation, has also been demonstrated. (S. Geetha, 2014). Ginger, for instance, is known for its anti-inflammatory and antioxidant properties, which have been attributed to its phenolic compounds like gingerol. Using assays such as the ORAC or DPPH can quantify its antioxidant potential. Ashwagandha, on the other hand, contains bioactive compounds like withanolides, which are associated with adaptogenic and anti-stress effects (Stanley Chukodizie *et al*., 2015). Assays like the TPC and FRAP can help determine its antioxidant potential. Milk thistle is known for its potential in liver health due to its silymarin content, and assays measuring liver enzymes or lipid peroxidation can shed light on its hepatoprotective potential.

Antioxidants protect our bodies by scavenging free radicals. They have been related to the prevention of

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cancer, atherosclerosis, heart disease, and kidney illness. Many tests have been devised to measure a substance's antioxidant activity. Here, the antioxidant activity was checked by performing four different assays. DPPH, ABTS, SO, and NO.

Raw ginger powder's DPPH assay's extract shows a higher level of scavenging activity (48.08%) than prepared ginger powder extract (38.78%). Ashwagandha extract exhibited (43.29%) inhibitory activity and milk thistle extract exhibited (56.35%). The combination of raw and prepared ginger exhibited (63.75%), whereas the combination of ashwagandha and milk thistle exhibited (49.23%).

Raw ginger powder extract from the ABTS experiment shows (73.65%) higher scavenging activity than prepared ginger powder extract (64.51%). Ashwagandha extract exhibited (69.73%) inhibitory activity and milk thistle extract exhibited (73.65%). The combination of raw and prepared ginger exhibited (63.75%), whereas the combination of ashwagandha and milk thistle exhibited (59.89%).

Raw ginger powder's scavenging activity in the Nitric Oxide Assay was lower than that of prepared ginger powder (38.3%), at (41.45%). Ashwagandha extract exhibited (43.21%) inhibitory activity and milk thistle extract exhibited (46.45%). The combination of raw and prepared ginger exhibited (46.63%), whereas the combination of ashwagandha and milk thistle exhibited (50.97%).

Compared to the extract of prepared ginger powder (33.9%), the Super Oxide assay results for the raw ginger powder extract show a higher scavenging activity of (39.67%). Ashwagandha extract exhibited (43.73%) inhibition activity and milk thistle extract exhibited (39.67%). Bibi Sadeer, *et al.*, 2020 recommended these assays. The combination of raw and prepared ginger exhibited (39.13%), whereas the combination of ashwagandha and milk thistle exhibited (45.86%). Natural antioxidants are preferred over synthetic antioxidants because they are less detrimental to the environment and are safer to ingest (Al-Dabbagh et al., 2018).

Over a broad spectral range, an FTIR spectrophotometer concurrently gathers high-resolution special data. The extracts of ginger, milk thistle, and ashwagandha were subjected to FTIR analysis, which revealed the existence of different bond or functional groups. In our raw ginger extract present functional groups were alcohol, alkane, phenols, nitro and fluro compound, carboxylic group, and aldehydes. The prepared ginger extract showed the presence of alcohol, aliphatic primary amine, cyclic alkene, anhydride, phenols, and aromatic compound. Extract of ashwagandha was positive in sulfoxide, alcohol, and fluro compound. Respectively, In the extract of milk thistle, present functional groups were alcohol, amine, alkene, sulfoxide, Alene, aromatic and nitro compounds, and fluoro compounds.

Hatched nauplii are distinct for the initial cytotoxicity investigation, according to the current study. Although there is another approach, inhibiting egg hatching (Carballo et al., 2002), that is comparable to the brine shrimp lethality experiment for marine natural products, it appears to be less sensitive in identifying the toxicity of macroalgae extracts. LC_{50} values of four plant extracts raw ginger, prepared ginger, Ashwagandha, and Milk thistle were determined and were found to be 28.33, 38, 46.66 and 20.22 µg/mL respectively. However, the combined extract of raw and prepared ginger, aswagandha, and silymarin had an LC50 of 36.75 and 42.95 g/mL.

5. CONCLUSION

The entire study has evidentially highlighted the effectiveness of plant extracts that include Ginger, Ashwagandha, and Milk thistle. Various parameters of plants have been used in this study to derive the actual potential of plant extracts and their characteristics to prevent diseases. The methodological interpretation of the study has been followed by the collection of plants sample and applying the Soxhlet technique to analyze the phytochemicals of the plants. Quantitative analysis of the photochemical also has been determined in this study with Total Phenolic Contents and Total Flavonoid Contents along with Total Tannin Contents.

ABTS, DPPH, NO and SO Assay have been conducted in this study to analyze the antioxidant capability of the plant extracts. The concept of Synergism also has been briefly discussed over here. A brief assessment of the antioxidant activity of the plant's extracts has been discussed in this study. In the discussion part, the study has interpreted the phytochemistry of the plants' extracts that highlights the carbohydrate, phenols, terpenoids,

flavonoids, quinones, glycosides, coumarins, and amino acid contents in the substances. The FTIR analysis interpreted the absorption or emission ability of the plant's characteristics. According to Clarkson's toxicity index, the extract in BSLA was determined to be both biologically active and non-toxic.The overall consequence of the study has justifiably highlighted the intense capability of particular plant extraction on medical grounds. According to the study's findings, extracts of plant's is a rich source of naturally occurring antioxidants and anticancer compounds that could be exploited as a possible therapeutic agent.

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