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COMPARISON OF INVITRO PHARMACOLOGICAL ACTIVITIES OF GREEN AND RED CULTIVAR OF ALTERNANTHERA SESSILIS FOR COMMERCIAL APPLICATION

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ABSTRACT

The present study aimed to investigate the pharmacognostical, phytochemical, and biological properties of the green and red cultivars of *Alternanthera sessilis* leaves. A systematic approach was followed, including authentication, morphological evaluation, successive solvent extraction, and phytochemical screening. Biological activities such as antioxidant, anti- inflammatory, and anti-obesity effects were assessed using in-vitro models. Pharmacognostical and morphological analyses confirmed the identity of the plant, while organoleptic evaluation supported standardization. Phytochemical screening revealed the presence of secondary metabolites like alkaloids, steroids, saponins, polyphenols, and flavonoids, which are associated with therapeutic effects. Antioxidant activity evaluated through DPPH and FRAP assays showed that the alcoholic extract of the red cultivar exhibited the highest free radical scavenging ability. Additionally, anti-obesity potential was observed via pancreatic lipase inhibition, indicating a possible role in reducing fat absorption and supporting weight management. These findings suggest that *Alternanthera sessilis*, particularly the red cultivar, is a promising natural source of antioxidants and anti-obesity agents, with its phytoconstituents likely responsible for the observed therapeutic effects.

KEYWORDS: *Alternanthera sessilis*, Antioxidant, Phytochemical Screening, Anti-inflammatory, Anti-obesity.

INTRODUCTION

Botanical description: *Alternanthera sessilis* is an annual or perennial herb belonging to the family Amaranthaceae. Commonly regarded as a noxious weed, it has been used as a food source since ancient times. The genus *Alternanthera* includes approximately 80–200 species, with an uncertain native range, though some reports suggest origins in North America and the tropical/subtropical forests of South America.[1] The plant grows 0.2–1 m tall and features strong creeping taproots. Its stems are prostrate, often rooting at the nodes, with a cylindrical, slightly hairy appearance and multiple erect branches. It reproduces through vegetative fragments and seeds, which are dispersed by ants (myrmecochory).[2]

Flowering and fruiting occur throughout the year in tropical regions such as India and Java. In India, peak vegetative growth aligns with the monsoon season, while reproductive growth is most vigorous post-monsoon. Flowers are self-pollinated, and fruits are dispersed by wind and water. Flowering seasons vary globally: May–July in China, July–September for fruiting, and summer to early autumn in North America.[3]

Vernacular names

- **Hindi**: Garundi, Guroo
- **Kannada**: Hona Gonne
- **Manipuri**: Phakchet
- **Marathi**: Kanchari
- **Tamil**: Ponnanganni
- **Malayalam**: Ponnankannikkira
- **Telugu**: Ponnagantikura.
- **Oriya**: Madaranga
- **Konkani**: Koypa
- **Sanskrit**: Matsyakshi
- **Nepali**: Bhiringi Jhaar

Figure 1: *Alternanthera sessilis*(green).

Taxonomical classification[4]

- **Kingdom**: Plantae
- **Subkingdom**: Trophobionts
- **Division**: Spermatophyta
- **Class**: Magnoliopsida
- **Subclass**: Caryophyllid
- **Order**: Caryophyllales
- **Family**: Amaranthaceae
- **Genus**: *Alternanthera*
- **Species**: *Alternanthera sessilis* Linn

Figure 2: Alternanthera sessilis(red)

Ethnomedical uses: *A. sessilis* is widely used in traditional medicine. It treats stomach disorders, diarrhoea, dysentery, and skin diseases. In Nigeria, the pounded plant is applied for headaches and vertigo. In Senegal and India, it is used for snakebites. In Taiwan, it's a key ingredient in herbal remedies for hepatitis, bronchitis, asthma, and lung ailments. In Thailand and Sri Lanka, it serves as a galactagogue. The plant also shows antisecretory, cytoprotective, and anthelmintic effects.[5] The juice, taken on an empty stomach, is traditionally used against intestinal worms.[6] Studies suggest ethanolic leaf extracts possess anticancer properties, especially for colon cancer.[7] Other uses include treatment for neuralgia, liver obstruction, leucorrhoea, gonorrhoea, and fever.[8,9]

Chemical constituents: *A. sessilis* contains a variety of bioactive compounds, including β- sitosterol, stigmasterol, campesterol, lupeol, α-spinasterol, cycloeucalenol, nonacosane, oxalic acid, esters, and hydrocarbons. GC-MS analyses by various researchers have identified over 50 major compounds. Katya Kini Muniandy et al. reported prevalent compounds such as 2,4- dihydroxy-2,5-dimethyl-3(2H)-furan-3-one, hexadecenoic acid, and phenyl-palmitate. Saipraasad Gothai et al. found alkanes, esters, flavonoids, terpenes, organic acids, and steroids. Khan et al. identified essential oil components such as hexa-methyl-trisiloxane derivatives, dioxolanes, and dimethoxyfuran derivatives in both flowers and leaves through GC-MS profiling. These constituents contribute to the plant's wide range of therapeutic applications.[10]

MATERIALS AND METHODOLOGY

Materials: The material such as Hydrochloric acid, Sulphuric acid, Chloroform, Ferric chloride, Benedict's reagent, Molisch reagent, Dimethyl Sulfoxide, Alcohol, Methanol, Ascorbic acid, Citric acid of pharma grade or the best possible laboratory was used as supplied by the manufacturers. All materials and instruments utilised in the work were sourced from various sources.

Methods: Collection and Authentication: Fresh leaves of green and red cultivars of *Alternanthera sessilis* were collected during June–July from a home garden in Maddur, Mandya, India. The plant material was identified and authenticated by a qualified botanist. After collection, the leaves were thoroughly washed, cut into small pieces, and shade-dried. The dried samples were then ground into coarse powder using a mechanical grinder, and a voucher specimen was preserved for future reference.

Successive solvent extraction of crude drugs using various solvents: Fresh, shade-dried leaves of green and red cultivars of *Alternanthera sessilis* were powdered and divided into six portions (10 g each). Each was macerated in 100 mL of one of three solvents: alcohol, hydroalcohol (70% alcohol:30% water), and hydroalcohol with 10% citric acid (70% alcohol:20% water:10% citric acid). The mixtures were kept at room temperature for seven days with occasional shaking, then filtered and concentrated under reduced pressure using a hot plate magnetic stirrer. The final extracts were stored in amber bottles under refrigeration and examined for yield, colour, and consistency.[11]

Anti-oxidant activity by DPPH assay: The DPPH free radical scavenging assay was performed to evaluate antioxidant activity based on the reduction of the stable violet-colored DPPH radical to a pale-yellow form upon hydrogen donation. A stock solution (1 mg/mL) of ascorbic acid (standard) and methanolic plant extract was prepared in DMSO, and aliquots of 15–75 µg were used. Each sample was mixed with 2.9 mL of methanolic DPPH solution, while the control contained DMSO and DPPH. Samples were incubated in the dark for 30 minutes, and absorbance was measured at 517 nm. The percentage

inhibition was calculated, and IC_{50} values were determined from the concentration vs. % inhibition plot.[12,13]

Preliminary phytochemical screening of extract.[14,15,16]: Screening of the above selected medicinal plant for various phytochemical constituents were carried out using standard methods as described in Table 1.

Table 1: Preliminary phytochemical tests for plant extract.

Phytoconstituents	Test	Observation
Tannins (Braymer's Test)	2ml extract + 2ml H2O + 2-3 drops FeCl3 (5%)	Green precipitate
Flavonoids	1ml extract + 1ml Pb(OAc)4 (10%)	Yellow coloration
Terpenoids	Terpenoids 2ml extract + 2ml (CH3CO)2O + 2-3 drops conc. H2SO4	Deep red coloration
Saponins (Foam Test)	5ml extract + 5ml H2O + heat 5ml extract + Olive oil (few drops)	Froth appears Emulsion forms
Steroids (Salkowski Test)	2ml extract + 2ml CHCl3 + 2ml H2SO4 (conc.)	Reddish brown ring at the junction
Carbohydrates (Molisch's Test)	2ml extract + 10ml H2O + 2 drops Ethanolic αnaphthol (20%) +2ml H2SO4 (conc.)	Reddish violet ring at the junction
Glycosides (Liebermann's Test)	2ml extract + 2ml CHCl3 + 2ml CH3COOH	Violet to Blue to Green coloration
Alkaloids (Hager's Test)	2ml extract + few drops of Hager's reagent	Yellow precipitate
Proteins (Xanthoproteic Test)	1ml extract + 1ml H2SO4 (conc.)	White precipitate

Anti-oxidant activity by FRAP assay: The FRAP assay measures antioxidant power based on the reduction of Fe^{3+} to Fe^{2+} by antioxidants, forming a blue-colored Perl's Prussian complex with maximum absorbance at 700 nm. In the assay, samples (ascorbic acid or plant extract) are mixed with phosphate buffer and potassium ferricyanide, incubated at 50°C for 30 minutes, cooled, and treated with TCA. After centrifugation, the supernatant is mixed with distilled water and ferric chloride, and the absorbance is measured at 700 nm. A higher absorbance indicates stronger antioxidant activity.[17]

Anti-inflammatory activity by egg albumin denaturation assay: The egg albumin denaturation assay evaluates anti-inflammatory activity by measuring a sample's ability to inhibit heat-induced protein denaturation. Egg albumin denatures upon heating, forming a turbid solution; anti-inflammatory agents reduce this turbidity by stabilizing the protein. In the assay, the test extract is mixed with 1% egg albumin, pH adjusted to 6.4, incubated at 37°C, then heated at 57°C for 30 minutes. After cooling, turbidity is measured at 660 nm. Lower absorbance indicates stronger anti-inflammatory potential. Aspirin serves as the standard, and appropriate controls are included for comparison.[18]

Anti-obesity activity by pancreatic lipase enzyme inhibition assay: The pancreatic lipase inhibition assay measures the ability of a test sample to inhibit lipase activity, which hydrolyzes p-nitrophenyl butyrate (p-NPB) into p-nitrophenol. In the assay, pancreatic lipase (0.1 mg/mL in 0.1 M Tris-HCl buffer, pH 8) is pre-incubated with the test sample at 37°C for 15 minutes. Then, p-NPB is added, and the reaction continues for another 15 minutes. The release of p- nitrophenol is measured at 405 nm. Orlistat is used as a positive control. Lipase inhibition is calculated based on changes in absorbance, indicating the test compound's inhibitory potential.[19]

RESULT

Successive solvent extraction of crude drugs using various solvents: The percentage yield of extracts obtained from successive solvent extraction of the leaves of Alternanthera sessilis given in the Table 2.

Table 2: The percentage yield of extracts obtained from successive solvent extraction.

Plant Name	Part Used	Method of Extraction	Solvents	Average Extractive Value (% w/w)
Red Alternanthera sessilis	Leaves	Maceration Process	Alcohol	8.1
			Hydro alcohol	12.4
			Hydro alcohol with 10% Citric acid	28.2
Green Alternanthera Sessilis	Leaves	Maceration Process	Alcohol	6.7
			Hydro alcohol	15.2
			Hydro alcohol with 10% Citric acid	17.2

Anti-oxidant activity by DPPH assay: Medicinal plants are an important source of antioxidants. Typical phenolic content that possesses antioxidant activity is known to be mainly phenolic acids and flavonoids. It is reported that the phenolic content is responsible for the variation in the antioxidant activity of the plant. Flavonoids are phenolic acids, which serve as an important source of antioxidants found in different medicinal plants and related phytomedicines. The anti-oxidant activity is due to their ability to reduce free radical.

Table 3: % Inhibition and IC50 value of ASR alcoholic extract.

Sample	Concentration (µg)	%RSA	IC_{50} Value (µg)
ASR (Alcoholic extract)	10	35.7326478	15.93154222
	15	50.8997429	
	20	59.7686375	
	25	67.8663239	
	30	72.7506427	

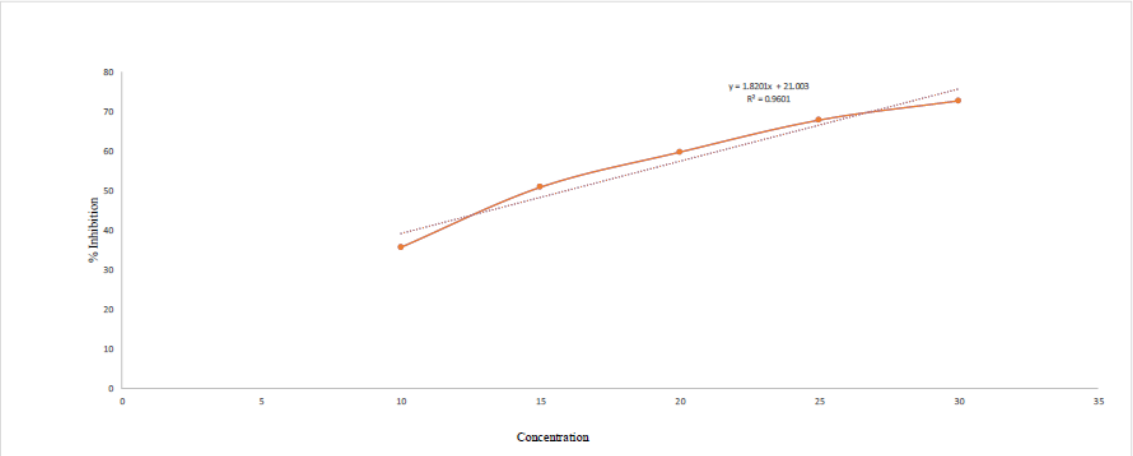

Figure 3: % Inhibition and IC50 value of ASR alcoholic extract.

Table 4: % Inhibition and IC50 value of ASR Hydro alcoholic extract.

Sample	Concentration (µg)	%RSA	IC_{50} Value (µg)
ASR (Hydro alcoholic extract)	10	17.8663239	27.87326733
	15	13.7532134	
	20	27.7634961	
	25	39.2030848	
	30	61.9537275	

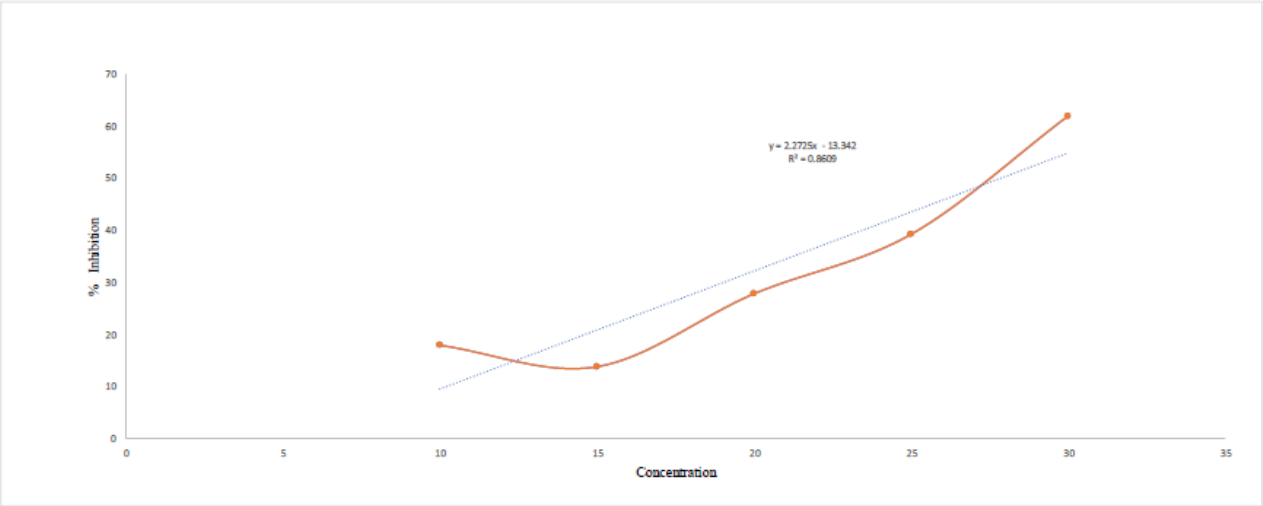

Figure 4: % Inhibition and IC50 value of ASR Hydro alcoholic extract.

Table 5: % Inhibition and IC50 value of ASR Hydro alcoholic + 10% citric acid.

Sample	Concentration (µg)	%RSA	IC50 Value (µg)
ASR (Hydro alcoholic + 10% citric acid)	10	10.7969152	41.69362487
	15	23.5218509	
	20	24.5501285	
	25	33.1619537	
	30	34.4473008	

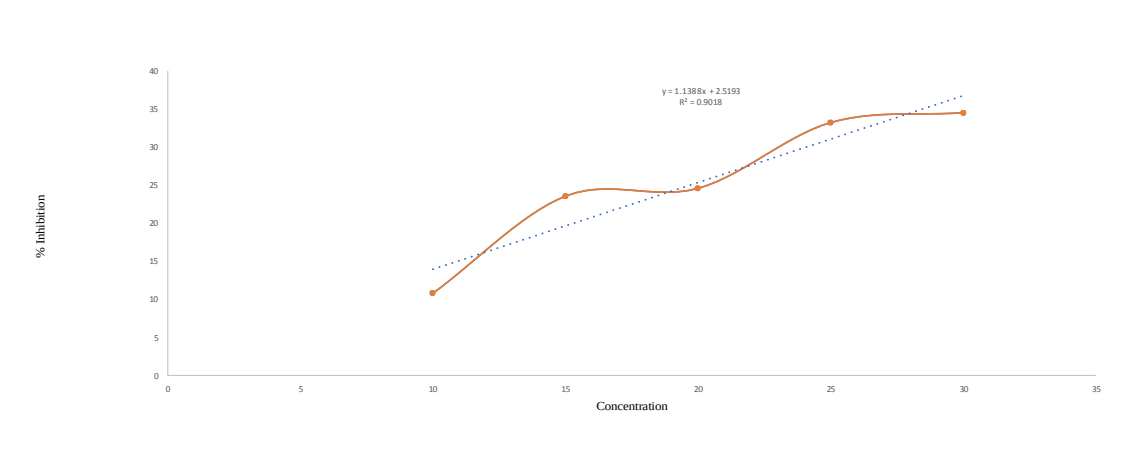

Figure 5: % Inhibition and IC50 value of ASR Hydro alcoholic + 10% citric acid.

Table 6: % Inhibition and IC50 value of ASG Alcoholic extract.

Sample	Concentration (µg)	%RSA	IC50 Value (µg)
ASG (Alcoholic extract)	10	15.1670951	105.8068966
	15	15.2956298	
	20	20.1799486	
	25	23.907455	
	30	19.9228792	

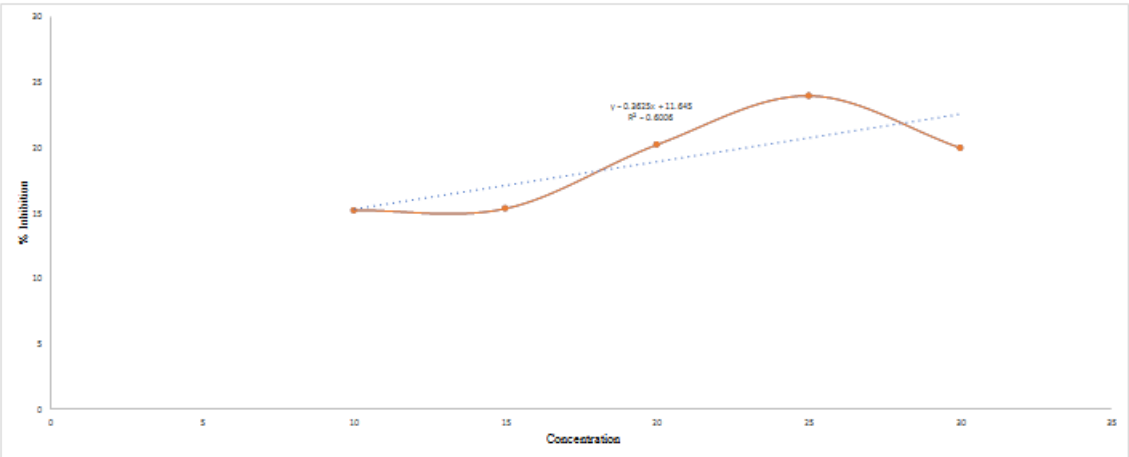

Figure 6: % Inhibition and IC50 value of ASG Alcoholic extract.

Table 7: % Inhibition and IC50 valve of ASG Hydro alcoholic extract.

Sample	Concentration (µg)	%RSA	IC50 Value (µg)
ASG (Hydro alcoholic extract)	10	7.84061697	37.71398506
	15	18.1233933	
	20	24.1645244	
	25	31.3624679	
	30	38.0462725	

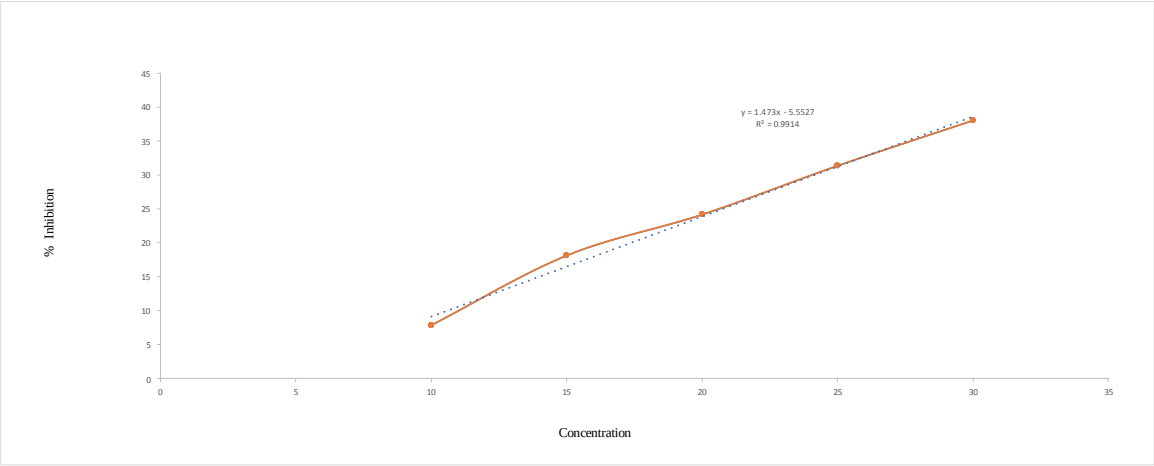

Figure 7: % Inhibition and IC50 valve of ASG Hydro alcoholic extract.

Table 8: % Inhibition and IC50 value of ASG Hydro alcoholic +10% citric acid.

Sample	Concentration (µg)	%RSA	IC_{50} Value (µg)
ASG (Hydro alcoholic + 10% citric acid)	10	20.437018	102.5911179
	15	21.2082262	
	20	21.9794344	
	25	24.6786632	
	30	26.8637532	

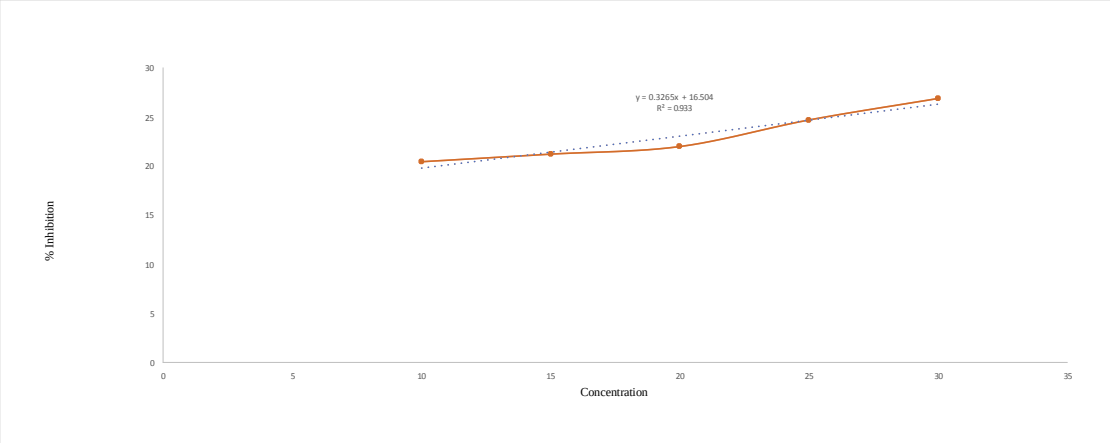

Figure 8: % Inhibition and IC50 value of ASG Hydro alcoholic +10% citric acid.

Preliminary phytochemical screening of extract: The alcoholic extracts of ASR (Red *Alternanthera Sessilis*) were screened for various tests mentioned in methodology.

The results are given in Table 9.

Table 9: Preliminary Phytochemical Screening of the Extract.

Sl no	Chemical Test	Alcoholic extracts of ASR
1	**Alkaloids**	
	Dragendroff's Test	Present
	Mayer's Test	Present
2	**Glycosides**	
	Keller-Kiliani Test	Absent
3	**Phytosterols and Triterpenoids**	
	Salkowski Test	Present
	Liebermann Burchard Test	Present
4	**Saponins**	

	Foam Test	Present
5	**Phenolic Compounds**	
	Ferric chloride	Present
6	**Flavonoids**	
	Shinoda Test	Present
	Ferric chloride Test	Present
7	**Carbohydrates**	
	Molisch's Test	Present
8	**Protein's & Amino acids**	
	Ninhydrin Test	Absent

Anti-oxidant activity by FRAP assay: The assay is based on the reduction of Fe^{3+} (ferric ion) to Fe^{2+} (ferrous ion) in the presence of antioxidant compounds. The reduced Fe^{2+} reacts with ferricyanide to form a Perl's Prussian blue complex, which has maximum absorbance at 700 nm. A higher absorbance indicates stronger reducing(antioxidant)power.

Table 10: FRAP Assay.

Sample	Concentration (µg)	Absorbance	%RSA
ASR (Alcoholic extract)	20	0.122	50.4065041
	40	0.129	47.5609756
	60	0.155	36.9918699
	80	0.162	34.1463415
	100	0.184	25.203252

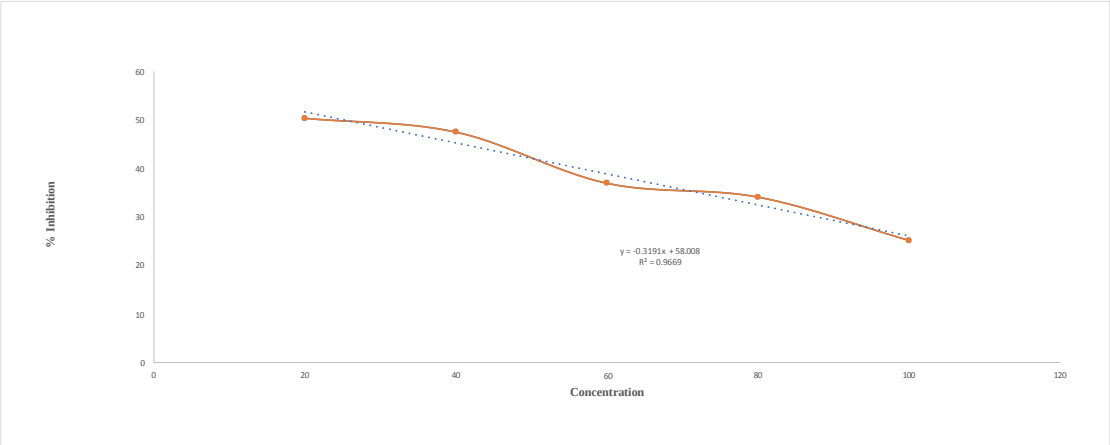

Figure 9: Anti-oxidant activity of ASR by FRAP assay.

Anti-inflammatory activity by egg albumin denaturation assay: Protein denaturation is a process where proteins lose their tertiary and secondary structure due to external stress, such as heat or chemicals, leading to loss of biological function. In this assay, egg albumin is used as a model protein to study heat-induced denaturation. When exposed to heat, egg albumin denatures and forms a turbid solution due to protein aggregation. Anti-inflammatory agents or plant extracts can inhibit this denaturation by stabilizing the protein structure. The extent of denaturation is measured as turbidity using a spectrophotometer at 660 nm. A lower absorbance indicates better protection against protein denaturation, reflecting potential anti-inflammatory activity of the test sample.

Table 11: Egg albumin assay.

Sample	Concentration (µg)	Absorbance	%RSA
ASR (Alcoholic extract)	20	0.086	-0.075
	40	0.087	-0.087
	60	0.088	-0.1
	80	0.089	-0.112
	100	0.103	-0.28

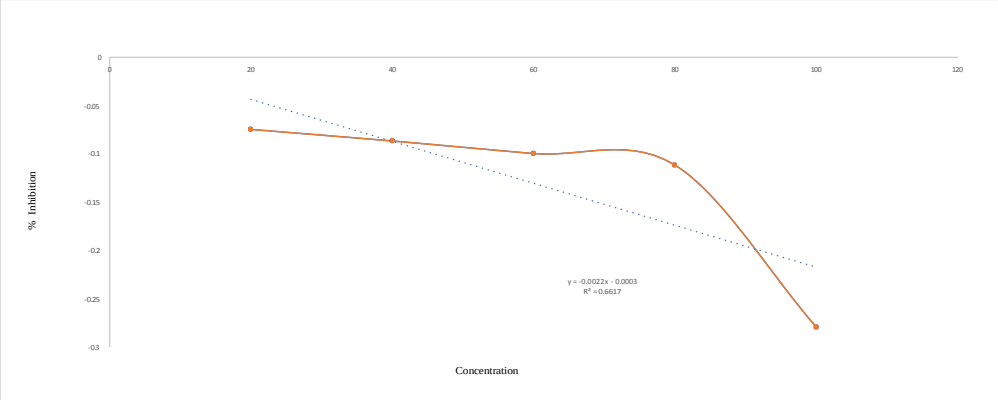

Figure 10: Anti-inflammatory activity of ASR by egg albumin assay.

Anti-obesity activity by pancreatic lipase enzyme inhibition assay: The principle of a pancreatic lipase enzyme inhibition assay is to measure the activity of pancreatic lipase and assess how specific inhibitors affect this activity. Pancreatic lipase is an enzyme that catalyses the hydrolysis of triglycerides into free fatty acids and glycerol in the digestive system. Inhibitors can interfere with the enzyme's activity either by competing with the substrate (competitive inhibition) or by binding to the enzyme or enzyme-substrate complex (non-competitive inhibition).

Table 12: Pancreatic Lipase enzyme inhibition.

Sample	Concentration (µg)	Absorbance	%RSA
ASR(Alcoholic extract)	20	0.361	17.7676538
	40	0.339	22.7790433
	60	0.303	30.9794989
	80	0.288	34.3963554
	100	0.250	43.0523918

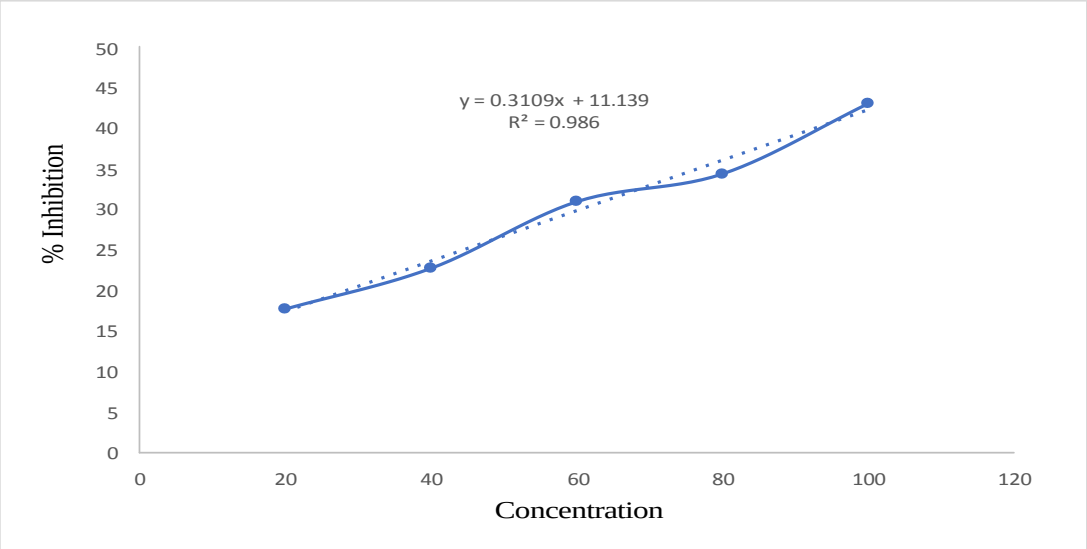

Figure 11: Anti-obesity activity of ASR by Pancreatic Lipase enzyme inhibition.

DISCUSSION

Successive solvent extraction of green and red cultivars of *Alternanthera sessilis* using alcohol, hydroalcohol, and hydroalcohol with 10% citric acid showed the highest yield in the red cultivar with the citric acid mixture. Antioxidant activity assessed through DPPH and FRAP assays indicated that alcoholic extracts, rich in phenolic compounds and flavonoids, had significant free radical scavenging and ferric reducing potential. Preliminary phytochemical screening confirmed the presence of various bioactive constituents including alkaloids, flavonoids, saponins, and glycosides, supporting their antioxidant and potential antiobesity activity. However, the extract did not exhibit anti-inflammatory effects in the egg albumin denaturation assay, possibly due to methodological limitations or alternate mechanisms of

action. Notably, the alcoholic extract showed significant anti-obesity potential through effective inhibition of pancreatic lipase enzyme activity, as demonstrated in the P-NPB hydrolysis assay.

CONCLUSION

The conclusion drawn from the various researches carried out in present study which includes various parameters used for identification of *Alternanthera sessilis* leaves and also detection of adulteration. This helps in proper identification of herb taken for the study. Natural products or herbal drugs are more acceptable with belief that they are safer or have fewer side effects than the synthetic ones. Herbal treatments have growing demand in the world market. In this study attempt has been made to develop and establish a safe tool for preliminary phytochemical, anti- oxidant and anti-obesity activity. Finally, the conclusion drawn from the study was, leaf extracts used for the study showed significant results towards In-vitro evaluation of anti-obesity activity and anti-oxidant activity. The alcoholic leaf extracts of *Alternanthera sessilis* was found to be more effective compare to other solvent extracts in exhibiting anti-oxidant activity.

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