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EVALUATION OF ANALGESIC AND ANTIOXIDANT ACTIVITY OF LEAVES EXTRACT OF XANTHOSOMA SAGITTIFOLIUM

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ABSTRACT

The present study investigates Xanthosoma sagittifolium leaf phytochemical composition, acute toxicity, analgesic characteristics, and antioxidant activities. Bioactive chemicals including alkaloids, anthraquinone glycosides, proteins, amino acids, carbs, saponins, flavonoids, phenols, and lipids were identified in the phytochemical study of the ethanolic leaf extract. There were no fatalities at dosages up to 2000 mg/kg in acute toxicity studies that followed OECD standards, indicating a safe LD50 within Category 4 (300-2000 mg/kg) of the Global Harmonized System (GHS). At 100, 200, and 300 mg/kg, the tail-flick method demonstrated significant analgesic effects that were dose-dependent for both the methanolic and ethanolic extracts. There was a notable uptick in antioxidant markers such as glutathione (GSH), superoxide dismutase (SOD), and catalase (CAT) levels after therapy, indicating that the treated groups experienced less oxidative stress. Research conducted here confirms that X. sagittifolium contains natural analgesic and antioxidant chemicals, lending credence to its long history of use in traditional medicine and suggesting that it may be useful in treating pain and diseases associated with oxidative stress.

1. INTRODUCTION

1.1 Harbal medicine and plants

The Rigveda contains the oldest records of medical plant use, indicating that early people recognized and employed natural substances to address various ailments for millennia. These medicinal plants have been integral to ancient medicine and continue to be vital to global healthcare systems today. Basso et al. (2005) noted that plants are a prolific source of bioactive chemicals owing to their chemical variety. Their role in Complementary and Alternative Medicine (CAM) is mainly due to their ability to generate secondary metabolites, which have.

1.2 The Role of Herbal Meds in Contemporary Healthcare

The incorporation of herbal medicine into mainstream healthcare is on the rise, thanks to scientific advancements. emphasizing the need for its integration with evidence-based practices. Some important factors are[13]:

To ensure that herbal products are consistently of high quality and potency, they must be standardized. Quality control involves identifying active compounds, ensuring appropriate concentrations, and testing for contaminants.[14]

Clinical Trials and Research: Modern research has validated the efficacy of various herbs. For instance, clinical trials on St. John's Wort show its efficacy in mild to moderate depression, and Ginger has been proven effective for nausea.[15]

2. MATERIAL AND METHODS

2.1 Identification, collection and authentication of plant

Leaves were chosen based on their historic applications, and a thorough review of existing literature was conducted to exclude any previous comparable research. A comprehensive literature review was conducted using several sources including books, internet resources, and the Medicinal and Aromatic Plants Abstracts (MAPA) database spanning from 1979 to 2018.

The leaves of Xanthosoma sagittifolium were gathered from Sardhana, Dist. Meerut, Uttar Pradesh. The medications were discovered and validated by Dr. Vijai Malik, Head of the Department of Botany at CCS University in Meerut, India.

2.2 Preparation of extract

The components of Xanthosoma sagittifolium were meticulously cleaned, segregated, and diced into little pieces. The goods were air-dried in the shade at room temperature for seven days. The dried plant material was coarsely pulverized using a mechanical grinder and then screened through a 40-mesh to ensure uniform particle size. The resultant powder was stored in a sealed jar to

preserve its purity for future use. Subsequently, this powdered substance was used to produce aqueous and other solvent-based extracts for further analysis.[57]

2.3 Preparation of Ethanolic extract of Xanthosoma sagittifolium

Petroleum ether (60–80°C, 2 L) and ethanol (2 L) used as solvents for the sequential Soxhlet extraction of 500 g of Xanthosoma sagittifolium aerial parts. The mixes were allowed to stand for seven days after the extraction method to get the crude extracts. A flash evaporator was used to concentrate the extracts to dryness, maintaining a controlled temperature range of 50–60°C and reduced pressure. The ethanol extract yielded 17.24% of a dark-hued extract, whereas the petroleum ether extract yielded 7.56% of a yellow-hued mass.[58]

2.4 Preparation of Methanolic extract of Xanthosoma sagittifolium

Soxhlet extraction was used to process Xanthosoma sagittifolium, resulting in a concentrated extract rich in bioactive compounds. During the 12-hour extraction procedure, 5.0 liters of methanol served as the solvent, including 500 grams of plant roots into the Soxhlet apparatus. Methanol was chosen due to its ability to dissolve several polar and semi-polar compounds likely present in the roots of Xanthosoma sagittifolium. Subsequent to the extraction technique, the extract was procured by heating it to a temperature range of 50 to 60 degrees Celsius in a water bath to remove the methanol solvent. The extract's thermolabile constituents remained intact due to the gentle heating, facilitating the evaporation of methanol. The extract's final yield of 20% indicates a substantial concentration of phytochemicals with possible therapeutic applications. This extraction procedure effectively isolates the active compounds from the roots, facilitating further pharmacological investigation and potential use in herbal medicine.[59]

2.5 Phytochemical analysis

An essential part of treating severe illnesses are phytochemicals, which are chemically active substances found in plants but do not provide nutrients. These compounds have distinct physiological effects on humans. Throughout history, these plants have been used to prevent and cure various ailments. The current research focused on the analysis of alkaloids, tannins, saponins, steroids, Anthraquinone glycosides, Cardiac Glycosides, and Flavonoids in the powder leaves of Trichosanthes dioica roxb. The analysis was conducted using the conventional techniques provided in references.[60]

2.6 Animal

Testing for acute toxicity, anti-inflammatory effects, and analgesic effectiveness was performed on albino rats (Wistar) weighing 150-200 g and Swiss albino mice weighing 20-25 g of both sexes. The animals were housed under regulated circumstances, including a 12-hour light-dark cycle, a temperature of 25°C ± 2°C, and a relative humidity of 60 ± 5%, sourced from Sri Venkateshwara Enterprises in Bengaluru. The animals were provided with water ad libitum and were given a regular pellet diet from Gold Mohur Lipton India Ltd. The subjects were stabilized for one week before the experiment, and the cage litter was changed thrice weekly to ensure cleanliness and comfort. The animals were managed delicately to alleviate stress. The studies adhered to CPCSEA rules (approval no. SCP/CPCSEA/P04/F120/2024), and ethical certification for the research was provided by the Institutional Animal Ethical Committee (IAEC) of SCP, Mangalore.

2.7 Screening method of Analgesic activity

a. By Tail-Flick Method

Five sets of albino mice were present, each including six animals. The tail flick test was performed 60 minutes post-administration of the respective pretreatment dosages of the drugs to the animals. For seven days, Group I, the control group, received 10 milliliters per kilogram of vehicle orally. Group II, serving as the positive control, received an intraperitoneal dose of 20 mg/kg of aspirin as a standard treatment for seven days. Over a period of seven days, Groups III, IV, and V received extracts of Xanthosoma sagittifolium at doses of 100, 200, and 300 mg/kg, respectively.

The reaction time was recorded at intervals of 0, 30, 60, 120, and 180 minutes post-drug administration using a tail flick analgesiometer. The temperature of the analgesiometer was maintained between 50 and 55°C throughout the experiment.[68]

b. Eddy's hot plate method

The analgesic effects of Xanthosoma sagittifolium extracts may be assessed in a well-organized manner. To fully understand how long it takes for the analgesic effect to kick in and how sensitive the hot plate method is to centrally acting analgesics, it's helpful to measure reaction durations at different intervals. A meaningful measure of the efficacy of each extract dose relative to the control and standard (Aspirin) treatments may be found by calculating the percentage change in response time.[69]

$$\% \text{ Increase Increase by reaction time} = [(R_t/Rc)-1]x100$$

Where,

R_t reaction time in treated group

R_c is reaction time in control group

2.8 Examination of antioxidant activity of Xanthosoma sagittifolium

a. Glutathione

Glutathione levels should be reliably assessed using this method. Since DTNB preferentially interacts with thiol groups to produce a quantifiable yellow color at 412 nm, it is a known method for detecting glutathione when used as a reagent. To minimize interference from extraneous components in the homogenate, the centrifugation and pH adjustment methods provide a steady reaction

environment that is clean.

The measurement was conducted using a reagent blank that lacked the tissue homogenate. Glutathione levels were quantified as nmol/mg protein, with the concentration derived from a standard curve established using standard glutathione solutions.[70]

b. Superoxide dismutase

This experiment checked if n-butanol inhibited the reduction of nitro-blue tetrazolium (NBT) to formazan blue at 560 nm. The blank control was used in this experiment. A total of 1.5 ml of reaction volume is produced by adding 50 µl of 186 µM phenazine methosulfate (PMS), 150 µl of 300 µM NBT, 0.4 ml of distilled water, and 0.6 ml of 0.052 M sodium pyrophosphate buffer (pH 8.3) to a 0.2 ml sample of tissue homogenate. This is done as part of the technique. After adding 0.1 ml of 780 µM NADH, the mixture was incubated at 30°C for 60 seconds to start the reaction. Following the incubation period, the reaction was stopped by adding 0.5 ml of glacial acetic acid. The formazan chromogen was then extracted using 2 ml of n-butanol. The mixture was centrifuged at 3000 rpm for an extra 10 minutes after a 10-minute standing break to help separate the butanol layer that contained the chromogen for analysis. A spectrophotometer was used to measure the absorbance of this layer at 560 nm, with n-butanol acting as the blank. To set a standard, an enzyme-free control reaction was performed. Milliunits per milligram of protein (mU/mg protein) is the unit of enzyme activity, which is defined as the concentration needed to induce 50% suppression of the chromogen's optical density at 560 nm under certain conditions. By measuring the inhibitory effect on superoxide-mediated NBT reduction, this method is often used to evaluate superoxide dismutase activity.[71]

c. Catalase

Gastric mucosal scrapings were homogenized at a concentration of 5% in M/150 phosphate buffer at a temperature of 1-4°C. The homogenate was then centrifuged, and the sediment was agitated with cold phosphate buffer. The mixture was let to remain in the cold with intermittent agitation, and the extraction procedure was conducted twice. The supernatants from each extraction were amalgamated for the test. Catalase activity was determined using the formula established by Aebi et al. (1975), with findings represented in milliunits per milligram of protein (mU/mg protein).[72]

Statistical Analysis

The data were presented as mean ± SEM (standard error of the mean). Statistical analysis was conducted using one-way analysis of variance (ANOVA), succeeded by Dunnett's test for post-hoc comparisons. A p-value below 0.05 ($P < 0.05$) was deemed statistically significant, indicating that the observed differences between groups were unlikely to be attributable to chance and represented a genuine impact of the therapy.

3. RESULTS AND DISCUSSION

3.1 PLANT AUTHENTICATION

Leaves were selected on the base of traditional uses and extensive literature survey was done to rule out similar work in the past. Literature survey was done from books, online sources and Medicinal and Aromatic Plants Abstracts (MAPA) from 1979-2018. The *Xanthosoma sagittifolium* were collected from Sardhana, Dist. Meerut, Uttar Pradesh. The drugs was identified and authenticated by Dr. Vijai Malik, HOD, Department of Botany, CCS University, Meerut, India.

Table 3.1: Result of Acute toxicity study.

Groups	No. of Animals	Dose (mg/kg)	Results
1	3	2000	No death
2	3	400	No death
3	3	400	No death

3.2 Evaluation of analgesic activity by tail-Flick methods

3.2.1 Analgesic activity of *Xanthosoma sagittifolium in methanolic extract*

Tabl 3.2: Analgesic activity of *Xanthosoma sagittifolium in methanolic extract.*

Groups	Dose	Mean reaction time in sec by tail-Flick method				
		0 min	30 min	60 min	2 hrs	3 hrs
Group I (Control)	5 (Ml/kg)	3.1±0.07	3.5±0.16	3.8±0.14	3.9±0.34	3.9±0.12
Group II (Positive Control)	20 (mg/kg)	3.1±0.09	5.8±0.14	5.5±0.24	4.7±0.17	3.7±0.19
Group III (MEXS)	100 (mg/kg)	3.1±0.12	5.5±0.11	5.3±0.17	4.6±0.11	3.8±0.16
Group IV (MEXS)	200 (mg/kg)	3.1±0.14	5.3±0.13	4.9±0.13	4.6±0.13	3.4±0.13
Group V (MEXS)	300 (mg/kg)	3.1±0.18	4.8±0.15	4.3±0.17	3.8±0.14	3.2±0.15

Values are shown as the mean plus standard error of the mean. There were six animals in each group. The number in parentheses indicates the increase in paw volume. A statistically significant result was determined by using ANOVA followed by Dunnett's test, with a significance level of $P < 0.05$.

MEXS= Methanolic Extract of Xanthosoma sagittifolium

3.2.2 Analgesic activity of Xanthosoma sagittifolium in Ethanolic extract

Table 3.3: Results of analgesic effect of Xanthosoma sagittifolium on rat.

Groups	Dose	Mean reaction time in sec by tail-Flick method				
		0 min	30 min	60 min	2 hrs	3 hrs
Group I (Control)	5 (ml/kg)	3.3±0.08	3.3±0.19	3.3±0.13	3.3±0.38	3.3±0.10
Group II (Positive Control)	20 (mg/kg)	3.6±0.10	7.5±0.67	14±0.27	16±0.19	20±0.17
Group III (EEXS)	100 (mg/kg)	6.5±0.18	11±0.19	14±0.15	16±0.10	17±0.10
Group IV (EEXS)	200 (mg/kg)	6.9±0.13	12±0.18	15±0.10	17±0.13	18±0.45
Group V (EEXS)	300 (mg/kg)	7.2±0.16	13±0.11	16±0.17	18±0.15	20±0.08

Values are shown as the mean plus standard error of the mean. There were six animals in each group. The number in parentheses indicates the increase in paw volume. A statistically significant result was determined by using ANOVA followed by Dunnett's test, with a significance level of $P < 0.05$.

EEXS= Ethanolic Extract of Xanthosoma sagittifolium

3.3 Antioxidant Activity

3.1.1 Glutathione

Table 3.4: Effect of *Xanthosoma sagittifolium extract on rat.*

Groups	Dose	SOD nM/mg protein	CAT nM/mg protein	GSH nM/mg protein
Group I (Control)	5 (ml/kg)	430.01±33.2	50.02±0.23	23.03±0.34
Group II (Positive Control)	20 (mg/kg)	545.05±0.34	70.07±0.32	34.56±1.23
Group III (EEXS)	100 (mg/kg)	592.56±45.2	120.35±35.2	33.54±0.23
Group IV (EEXS)	200 (mg/kg)	610.23±34.6	126.31±76.3	34.24±0.56
Group V (EEXS)	300 (mg/kg)	721.23±12.54	130.34±34.2	36.23±0.24

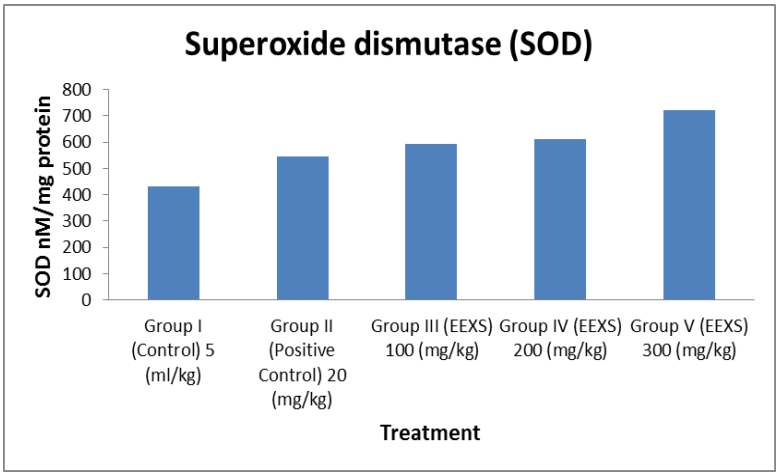

Figure 3.1: Results of Ethanolic Extract of Xanthosoma sagittifolium on superoxide dismutase on experimental rats.

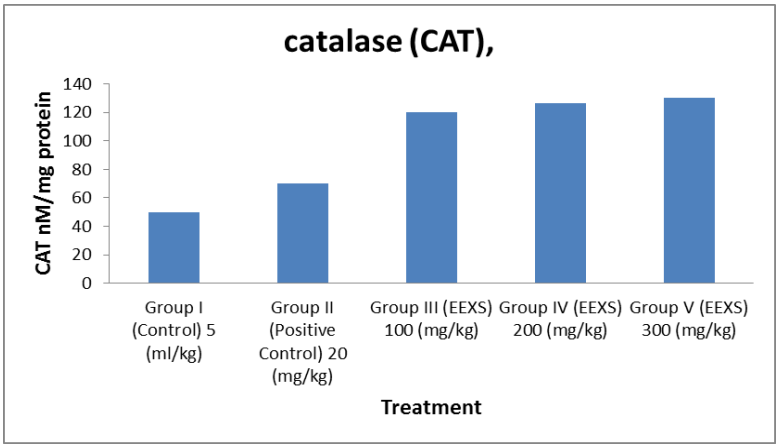

Figure 3.2: Results of Ethanolic Extract of Xanthosoma sagittifolium onCatalase (CAT) on experimental rats.

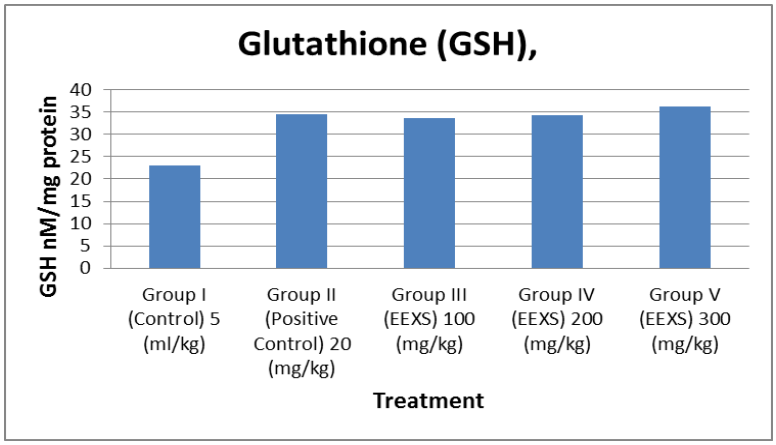

Figure 3.3: Results of Ethanolic Extract of Xanthosoma sagittifolium of glutathione (GSH), on experimental rats.

CONCLUSION

Xanthosoma sagittifolium's phytochemical profile, safety profile, and pharmacological potential have been greatly illuminated by this study. Results from phytochemical testing on the ethanolic leaf extract confirmed the presence of numerous bioactive compounds, including lipids, proteins, amino acids, carbohydrates, saponins, flavonoids, phenols, and anthraquinone glycosides. This suggests a rich source of medicinally useful substances. With no fatalities reported at doses up to 2000 mg/kg in acute toxicity testing, the extract is placed in Category 4 of the Globally Harmonized System.

The tail-flick method was used to measure the analgesic efficacy of X. sagittifolium extracts. Both the methanolic and ethanolic extracts significantly extended the time it took for experimental participants to respond, but the ethanolic extract was more effective, especially at higher doses. The mice that were given the treatment showed a significant improvement in their antioxidant capacity, with levels of SOD, CAT, and GSH all rising. This helped to reduce the negative impact of oxidative stress. Xanthosoma sagittifolium has a long history of usage for pain, inflammation, and diseases associated with oxidative stress; this study adds credence to that use by demonstrating the plant's powerful antioxidant and analgesic properties. Based on these findings, X. sagittifolium should be further studied as a potential natural resource for the development of effective and safe therapeutics.

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