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PRELIMINARY INVESTIGATION OF BIOACTIVE CONSTITUENTS AND EVALUATION OF ANTIPYRETIC ACTIVITY OF ETHANOLIC EXTRACT OF AGAVE SISALANA

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Abstract:
Pyrexia or fever is caused as a secondary impact of infection, malignancy or other diseased states. It is the body's natural defense to create an environment where infectious agent or damaged tissue cannot survive. In present study, the antipyretic activity of ethanol extracts from Agave sisalana was evaluated using Brewer's yeast induced pyrexia in rats. The results showed that the ethanolic extract of Agave sisalana possesses a significant antipyretic effect in maintaining normal body temperature and reducing yeast-induced elevated body temperature in rats in a dose dependent manner and its effect is comparable to that of the standard antipyretic drug paracetamol. It was therefore concluded that the extracts of Agave sisalana demonstrated antipyretic activity, the ethanol extract was found to be potent than the aqueous extract.

Key words: *Agave sisalana, Ethanolic extract, Antipyretic activity.*

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INTRODUCTION:

Ayurvedic medicines mainly based on plants enjoy a respective position today, especially in the developing countries, where modern health services are limited. Safe effective and inexpensive indigenous remedies are gaining popularity among the people of both urban and rural areas especially in India and China. Information from ethnic groups or indigenous traditional medicines has played vital role in the discovery of novel products from plants as chemotherapeutic agents. Herbal medicines have been main source of primary healthcare in all over the world. From ancient times, plants have been catering as rich source of effective and safe medicines. About 80 % of world populations are still dependent on traditional medicines. Herbal medicines are finished, labeled medicinal products that contain as active ingredients, aerial or underground part of plants or other plant materials, or combination thereof, whether in the crude state or as plant preparations. Medicines containing plant materials combined with chemically defined active substances, including chemically defined isolated constituents of plants are not considered to be herbal medicines[1].

Herbal medicines continue to be a major market in US pharmaceuticals and constitute a multi-billion dollar business. Approximately 1500 botanicals are sold as dietary supplements; formulations are not subject to Food and Drug Administration (FDA) clinical toxicity testing to assure their safety and efficacy. The Indian herbal drug market size is about $1 billion and the export of plant based crude drug is around $100 million. The current market potential of herbal medicine is estimated about $ 80-250 billion in Europe and USA[2].

Pyrexia or fever is caused as a secondary impact of infection, malignancy or other diseased states. It is the body's natural defense to create an environment where infectious agent or damaged tissue cannot survive[3]. Normally the infected or damaged tissue initiates the enhanced formation of proinflammatory mediator's (Cytokines like interleukin 1β, α, β and TNF- α), which increase the synthesis of prostaglandin E2 (PG E2) near peptic hypothalamus area and thereby triggering the hypothalamus to elevate the body temperature[4]. *Agave sisalana* Perrine, popularly known as sisal, belonging to the Agavaceae family and is a monocotyledonous plant from Mexico. It is well adapted to the semiarid region of Northeast Brazil. Brazil is the world's largest producer of *A. sisalana* for the supply of the sisal fiber, and the sisal culture is one of the main economic activities in the semi-arid Bahia State, which accounts for about 90% of its production. This study aimed to determine antipyretic potential of ethanolic extract of *Agave sisalana* leaves.

MATERIAL AND METHODS:

Collection of plant material:

The plants have been selected on the basis of its availability and folk use of the plant. Leaves of *Agave sisalana* were collected from local area of Bhopal in the month of January, 2022. Drying of fresh plant parts were carried out in sun but under the shade. Dried leaves of *Agave sisalana* were preserved in plastic bags and closed tightly and powdered as per the requirements.

Extraction procedure

Following procedure was adopted for the preparation of extract from the shade dried and powdered herbs [5-6]:

Defatting of plant material

Leaves of *Agave sisalana* was shade dried at room temperature. The shade dried plant material was coarsely powdered and subjected to extraction with petroleum ether by soxhlet extraction. The extraction was continued till the defatting of the material had taken place.

Extraction by soxhlet extraction

60.5 gm of dried powdered leaves of *Agave sisalana* has been extracted with ethanolic solvent using soxhlet extraction process for 24-48 hrs, filtered and dried using vacuum evaporator at 40°C.

Phytochemical Screening:

Phytochemical screening: Phytochemical examinations were carried out for all the extracts as per the standard methods[7].

1. **Detection of alkaloids:** Extracts were dissolved individually in dilute Hydrochloric acid and filtered.

Mayer's Test: Filtrates were treated with Mayer's reagent (Potassium Mercuric Iodide). Formation of a yellow coloured precipitate indicates the presence of alkaloids.

Wagner's Test: Filtrates were treated with Wagner's reagent (Iodine in Potassium Iodide). Formation of brown/reddish precipitate indicates the presence of alkaloids.

Dragendroff's Test: Filtrates were treated with Dragendroff's reagent (solution of Potassium Bismuth Iodide). Formation of red precipitate indicates the presence of alkaloids.

Hager's Test: Filtrates were treated with Hager's reagent (saturated picric acid solution). Presence of alkaloids confirmed by the formation of yellow coloured precipitate.

2. **Detection of carbohydrates:** Extracts were dissolved individually in 5 ml distilled water and

filtered. The filtrates were used to test for the presence of carbohydrates.

Molisch's Test: Filtrates were treated with 2 drops of alcoholic α-naphthol solution in a test tube. Formation of the violet ring at the junction indicates the presence of Carbohydrates.

Benedict's Test: Filtrates were treated with Benedict's reagent and heated gently. Orange red precipitate indicates the presence of reducing sugars.

Fehling's Test: Filtrates were hydrolysed with dil. HCl, neutralized with alkali and heated with Fehling's A & B solutions. Formation of red precipitate indicates the presence of reducing sugars.

3. Detection of glycosides: Extracts were hydrolysed with dil. HCl, and then subjected to test for glycosides.

Legal's Test: Extracts were treated with sodium nitropruside in pyridine and sodium hydroxide. Formation of pink to blood red colour indicates the presence of cardiac glycosides.

4. Detection of saponins

Froth Test: Extracts were diluted with distilled water to 20ml and this was shaken in a graduated cylinder for 15 minutes. Formation of 1 cm layer of foam indicates the presence of saponins.

Foam Test: 0.5 gm of extract was shaken with 2 ml of water. If foam produced persists for ten minutes it indicates the presence of saponins.

5. Detection of phenols

Ferric Chloride Test: Extracts were treated with 3-4 drops of ferric chloride solution. Formation of bluish black colour indicates the presence of phenols.

6. Detection of tannins

Gelatin Test: To the extract 1% gelatin solution containing sodium chloride was added. Formation of white precipitate indicates the presence of tannins.

7. Detection of flavonoids

Alkaline Reagent Test: Extracts were treated with few drops of sodium hydroxide solution. Formation of intense yellow colour, which becomes colourless on addition of dilute acid, indicates the presence of flavonoids.

Lead acetate Test: Extracts were treated with few drops of lead acetate solution. Formation of yellow colour precipitate indicates the presence of flavonoids.

8. Detection of proteins

Xanthoproteic Test: The extracts were treated with few drops of conc. Nitric acid. Formation of yellow colour indicates the presence of proteins.

9. Detection of diterpenes

Copper acetate Test: Extracts were dissolved in water and treated with 3-4 drops of copper acetate solution. Formation of emerald green colour indicates the presence of diterpenes.

Quantitative estimation of bioactive compound:

Estimation of total phenolic content:

The total phenolic content of the extract was determined by the modified Folin-Ciocalteu method[8]. 10 mg Gallic acid was dissolved in 10 ml methanol, various aliquots of 5- 25µg/ml was prepared in methanol. 10mg of dried extracts of were dissolved in 10 ml methanol and filter. Two ml (1mg/ml) of this solution was used for the estimation of phenol. 2 ml of each extract or standard was mixed with 1 ml of Folin-Ciocalteu reagent (previously diluted with distilled water 1:10 v/v) and 1 ml (7.5g/l) of sodium carbonate. The mixture was vortexed for 15s and allowed to stand for 15 min for colour development. The absorbance was measured at 765 nm using a spectrophotometer.

6.5.2 Estimation of total flavonoids content:

10 mg quercetin was dissolved in 10 ml methanol, and various aliquots of 5- 25µg/ml were prepared in methanol. 10mg of dried extracts of were dissolved in 10 ml methanol and filter. Three ml (1mg/ml) of this solution was used for the estimation of flavonoid. 1 ml of 2% $AlCl_3$ methanolic solution was added to 3 ml of extract or standard and allowed to stand for 15 min at room temperature; absorbance was measured at 420 nm.

***In vivo* antipyretic activity of *Agave sisalana* extract:**

Animals:

Animal's Wistar strain albino rats of either sex weighing between 140– 160 g were used in the present study. They were provided normal diet and tap water ad labium and were exposed to 12-h light and 12-h dark cycle. The animals were acclimatized to the laboratory conditions before experiments. Experimental protocol was approved by Institutional Animal Ethics Committee. Care of the animals was taken as per guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Environment and Forests, Government of India. Experiment protocol was approved by Institutional Animal Ethics Committee.

Acute toxicity:

Five groups of five rats each were used. The test was conducted according to the method of, where rats received a single dose of the graded doses of the test extract[9]. The initial body weights of the rats were recorded. The control (group 1) received distilled water (5 ml/kg body weight), and groups 2 to 5 received Ethanolic extract of *Agave sisalana* at doses 50, 100, 200 and 400 mg/kg body weight of the rat

per os by means of a bulbed steel needle, respectively.

Observations were made for any physiological and behavioural changes that included feeding behaviour, increased or decreased activity due to drug reaction, stress and rat mortality. The rats were observed continuously for 3 h soon after administering the extract, then hourly for 72 h.

Antipyretic activity was measured by slightly modifying the method described by[10]. Prior to the experiment, the rats were maintained in separate cages for 7 days and the animals with approximately constant rectal temperature were selected for the study. Pyrexia was induced by subcutaneously injecting 15% w/v brewer's yeast suspension (10 mL/kg) into the animal's dorsum region. 18 h after the injection, the rectal temperature of each rat was measured using a digital thermometer. Only rats that showed an increase in temperature at 0.7 ℃ were used for experiments.

Grouping of animals:
The rats were divided into four groups of six animals each. The first group was treated as control whereas the second group as standard. Group III and IV were named as dose 1 and dose 2 respectively. Paracetamol (150 mg/kg) was administered orally to the rats of the second group. The Ethanolic extract of *Agave sisalana* in doses of 200 and 400 mg/kg was also administered orally to the rats of third and fourth group respectively. The rectal temperatures of all the rats were measured at 1, 2, 3, 4 and 5 h after drug administration and were compared with the control and standard groups.

Standardization of yeast-induced antipyretic model for the present study
Fever can be induced in experimental animals by intravenous or subcutaneous injection of pyrogens. To evaluate the antipyretic activity of test drugs, the most commonly employed method to induce fever involves injection of lipopolysaccharides (LPS) or brewer's yeast in rabbits or rats [11].

The animals were fasted for 18 h before the commencement of the experiment, but drinking water was provided ad libitum. Rectal temperature (TR) was measured by inserting a lubricated thermostat probe (external diameter 6 mm) 3 cm into the rectum of the animal. The probe was linked to a digital device, which displayed the temperature.

The test drugs and reference standard were administered to the respective groups. One hour after drug administration, the yeast injection was given s.c. and the rectal temperature was recorded up to the end of 5th hours. The rectal temperature of the control groups (yeast control) was compared with rectal temperature of the rats administered the test drugs.

Statistical analysis:
The results are presented as mean ± SEM. The difference between the groups was statistically analyzed using the unpaired Student's t test and analysis of variance (ANOVA) followed by Dunnett's t test for all the treated groups (except reference standard group, where only the unpaired Student's t test was applied). $P<.05$ was considered statistically significant. The level of significance was noted and interpreted accordingly.

RESULTS AND DISCUSSION:

The crude extract so obtained after the soxhlet extraction process, extract was further concentrated on water bath evaporation the solvent completely to obtain the actual yield of extraction. To obtain the percentage yield of extraction is very important phenomenon in phytochemical extraction to evaluate the standard extraction efficiency for a particular plant, different parts of same plant or different solvents used. The yield of extract obtained from samples using hydroalcoholic as solvent is depicted in the table 1. A small portion of the dried extracts were subjected to the phytochemical test using Kokate (1994) methods to test for alkaloids, glycosides, saponins, flavonoids and phenol separately for extracts of all samples. Small amount of each extract is suitably resuspended into the sterile distilled water to make the concentration of 1 mg per ml. The outcomes of the results are discussed separately in the table 2. The content of total phenolic compounds (TPC) content was expressed as mg/100mg of gallic acid equivalent of dry extract sample using the equation obtained from the calibration curve: $y = 0.035x + 0.015$, $R^2= 0.999$, where X is the gallic acid equivalent (GAE) and Y is the absorbance. The content of total flavonoid compounds (TFC) content was expressed as mg/100mg of quercetin equivalent of dry extract sample using the equation obtained from the calibration curve: $\mathbf{y = 0.031x - 0.000}$, $R^2= 0.999$, where X is the quercetin equivalent (QE) and Y is the absorbance table 3. Five groups of five rats each were used. The test was conducted according to the method of, where rats received a single dose of the graded doses of the test extract[65]. The initial body weights of the rats were recorded. The control (group 1) received distilled water (5 ml/kg body weight), and groups 2 to 5 received Ethanolic extract of *Agave sisalana* at doses 50, 100, 200 and 400 mg/kg body

weight of the rat per os by means of a bulbed steel needle, respectively table 4. Observations were made for any physiological and behavioural changes that included feeding behaviour, increased or decreased activity due to drug reaction, stress and rat mortality. The rats were observed continuously for 3 h soon after administering the extract, then hourly for 72 h.

Table 1: % Yield of leaves of *Agave sisalana*

S. No.	Extract	% Yield (w/w)
1.	Hydroalcoholic	7.56%

Table 2: Phytochemical screening of extract *of Agave sisalana*

S. No.	Constituents	Hydroalcoholic extract	Observation
1.	**Alkaloids**		
	Mayer's Test:	-ve	Green coloured
	Wagner's Test:	-ve	Green coloured
	Dragendroff's Test:	-ve	Light Green coloured
	Hager's Test:	+ve	Yellow coloured precipitate.
2.	**Glycosides**		
	Legal's test	+ve	Red coloured
3.	**Flavonoids**		
	Lead acetate	+ve	Yellow coloured precipitate
	Alkaline Reagent Test:	-ve	Yellow coloured
	Phenolics		
4.	Ferric Chloride Test	+ve	Black coloured
5.	**Proteins**		
	Xanthoproteic test	-ve	Green coloured
6.	**Carbohydrates**		
	Molisch's Test:	-ve	Yellow coloured
	Benedict's Test:	-ve	Yellow coloured
	Fehling's Test:	+ve	Red precipitate
7.	**Saponins**		
	Froth Test:	+ve	Layer of foam
	Foam Test:	-ve	No foam
8.	**Diterpins**		
	Copper acetate test	-ve	Sky blue coloured
9.	**Tannins**		
	Gelatin Test:	+ve	White precipitate

Table 3: Total phenolic and total flavonoid content of *Agave sisalana*

S. No.	Total Phenol content	Total flavonoid content
1.	0.586 mg/100mg	0.874 mg/100mg

Table 4: Effect of Ethanolic extract of *Agave sisalana* (EEAS) on Brewer's yeast-induced pyrexia in rats (mean ± SEM, n=6)

Treatment	Dose (mg/kg)	Rectal temperature (℃)						
		-18 h	0 h	1 h	2 h	3 h	4 h	5 h
Control	-	37.35±0.13	39.38±0.17	39.47±0.14	39.41±0.18	39.13±0.12	39.20±0.13	39.05±0.17
Paracetamol	150	37.28±0.30	39.18±0.23	38.85±0.20*	38.68±0.21*	38.26±0.25***	38.06±0.23**	37.68±0.24***
EEAS	200	36.18±0.24**	38.53±0.28**	38.34±0.31**	38.18±0.25***	37.21±0.20***	37.45±0.28***	37.10±0.30***
EEAS	400	37.95±0.32	39.43±0.29	39.09±0.27	38.75±0.30*	38.27±0.26**	38.14±0.33**	38.08±0.29**

Significantly different at * P<0.05, ** P<0.01, *** P<0.001 when compared to control

CONCLUSION:

The results showed that the ethanolic extract of *Agave sisalana* possesses a significant antipyretic effect in maintaining normal body temperature and reducing yeast-induced elevated body temperature in rats in a dose dependent manner and its effect is comparable to that of the standard antipyretic drug paracetamol. Furthermore, the significant reduction of yeast provoked elevated temperature of the tested animals by the extract at 200 mg/kg dose and 400 mg/kg of fractions appears to be due to the action of ursolic acid, β-sitosterol and its glucoside alone or in combination. In general, non-steroidal anti-inflammatory drugs produce their antipyretic action through the inhibition of prostaglandin synthetase within the hypothalamus[72]. Therefore, the antipyretic activity of ethanolic extract of *Agave sisalana* is probably by inhibition of prostaglandin synthesis in hypothalamus. The study concludes that antipyretic activities of ethanolic extract of *Agave sisalana* can also be due to the presence of alkaloids, phenol and flavonoids.

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