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Formulation and Evaluation of Added Sugarcane Jaggery Based Antioxidant and Fibrinolytic

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Abstract
Herbal drugs are highly rich in antioxidant properties. The oldest sweetener jaggery made from sugarcane (*Saccharum officinalis*) juice added with different extracts of herbal drugs was prepared and evaluated on different parameters *in vitro*. Pharmacognostical studies of individual herbal drugs and different formulations were performed and *in vitro* antioxidant activity was evaluated on DPPH assay. Sugarcane jaggery contains highly nutritive value with medicinal values. The jaggery contains vitamins, proteins and minerals which are essential constituents of body. Today the Ayurvedic industry prepares therapeutic formulation with jaggery for the treatment of various diseases and disorders.

Keywords
Jaggery, Sugarcane, Antioxidant, Medicinal herbs, Added jiggery, fibrinolytic

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INTRODUCTION
Sugarcane, member of grass family, has potential to grow upto 4.5m high under tropical conditions [(1)]. Sugarcane which belongs to the genus Saccharum has six species namely *S. officinarum, S. barberi, S. sinense, S. robustum, S. sponteneum and S. elude* [(2)]. Sugarcane is the vital raw material to a wide range of agro-processing industries to produce sugar, jaggery, khandsari and other industrial products. India is the largest consumer and second largest producer of sugar in the world [(3)]. Black pepper (*Piper nigrum* L.), is a flowering vine in the family Piperaceae that is native to South India. This spice has a profound impact on the Indian spice export landscape, contributing significantly to the country's economy [(4)]. As one of the oldest spices known to humanity, black pepper has a rich history of use those dates back to prehistoric times. The fruit of black pepper is a drupe, which is a berry-like structure known as a peppercorn [(5)]. Extract of black pepper contains tannin, saponin, total flavonoid in fruit is approximately (1.728 ± 0.049) mg, essential oil, Chavicine, Piperine[(6)]. Ginger (*Gingiber officinalis*) belongs to the order Zingiberales. This family features a variety of perennial aromatic plants that are characterized by their horizontal rhizomes, which serve as underground stems [(7)]. Ginger extracts are rich in a variety of bioactive compounds, such as gingerols, and other secondary metabolites are saponins, tannins, cardiac glycosides, carbohydrates, proteins which have been confirmed to exhibit a range of beneficial health effects. Ginger has anti-inflammatory activity, antioxidants, anti-hyperlipidemia, anticancer, anti-arrhhythemic, anti-obesity and antidiabetic properties [(8)]. *Piper longum* Linn. is popular as long pepper which belongs to family piperaceae. Plant is widely distributed in sub-tropical and the tropical regions in the world, mostly in Indian subcontinent, Middle Eastern countries, Sri Lanka, and the Americas. P. longum fruit contains piperine as the principle and active phyto-constituent, the piperine content in fruit is about 3-

5% [9]. The fruits possess starch, proteins and volatile oils, alkaloids, saponins, and carbohydrates etc. The black color fruits of P. longum are used as a stomachic, aboartifacient, pungent, laxative, anti-diarrhoeal, anti-bronchitis, abdominal complaints, aphrodiasic, stomachicanti-dysenteric, liver tonic, in urinary discharges, tumours, leprosy, insomnia, jaundice, and hiccoughs anti-asthmatic, diseases of the spleen, pains, inflammation etc[10]. The roots of P. longum are also used for the treatment of cardiovacular diseases. P. longum have several pharmacological properties like antibacterial, antifungal, insecticidal, hepatoprotective, adulticidal, antidepressant, anticancer, antiplatelet, antiamoebic, antiulcer, anti-obesity, anti-asthmatic larvicidal, etc. Ashwagandha (*Withania somnifera Linn)*, is medicinal herbs in *Ayurveda* used from ancient time [11]. All Ayurvedic class of literatures like *Susrutha, Charaka, Vagbhatta* and *nighantus* has mentioned the properties, its action and therapeutic uses. *Charaka, Vagbhatta* and *Susrutha* not reports about the *shukrala* properties or *vajeekarana* properties of *Ashwagandha*. Ashwagandha stem contains steroidal lactones like withanolides. The roots of Ashwagandha contains alkaloids, -sitesterol, polyphenols, 18 fatty acids, beta and phytosterols. Shatavari (*Asparagus racemosus*) is a medicinal plant which reported as 'rasayana' in Ayurveda which means drugs promoting overall well-being of body by increasing cellular resistance and vitality [12]. It is an Ayurvedic plant, known for its activities like hyperlipidemia, angina, dysmenorrhea, hypertension, cough, benign prostatic hyperplasia (BPH), leucorrhoea anxiety disorders, and urinary tract infections. The plant root contains large no of secondary metabolites like steroids, dihydrophenanthrene derivatives, flavonoids, furan derivatives, alkaloids, and essential oils. The length of roots of Shatavari is about 5-12 cm long and 1-2 cm in diameters. These are white or light brown in color externally and white internally. The plant roots possess various therapeutic applications like antioxidant, antidepressant, antiepileptic, diuretic, antitussive, hepato-protective, cardio-protective, anti-HIV, immunostimulant, antiulcerative, antibacterial, neurodegenerative [13].

METHODS

Requirements: DPPH, ascorbic acid and other chemicals used in study were analytical grade and were purchased from HiMedia. Shatavari root, Ashwagandha stem, dried ginger, long pepper (fruit), and black pepper fruit was collected from the local registered shop. Sugar cane juice of *Sacchrum officinarum* was collected from the local vender.

Morphology of drugs

1. **Shatavari:** Tuberous root, white to brownish and fleshy, spindle shape, swollen in middle
2. **Ashwagandha:** The roots of Ashwagandha are cylindrical, straight, unbranched, 10-15 cm in length, light brown in color.
3. **Dried Ginger:** Rhizomes are irregular thick in shape and branched. Light brown in color.
4. **Long Pepper:** Fruit also called as pippali, avoid drupe, 3-5 cm long cylindrical, blackish green in color.
5. **Black Pepper:** Fruit also called as peppercorns, 5 mm in diameters with rough surface, reddish black in color.

Microscopic study of crude drugs

Shatavari (root), ashwagandha (stem), dried ginger (rhizome), black pepper (fruit), long pepper (fruit) was collected from authorized registered supplier. All drugs were pulverized to make the course powder and the course powder of all drugs was used for microscopic study.5 gm from each drug course powder of all crude drugs was taken in separate test tube with chloral hydrate and heat till boil. After boiling filter the residue of each test tube and filtered residue was further used for microscopic study. Small portion of each residue was taken in separate watch glass and separately treated with chemicals for microscopic study. The slides were prepared and observed the presence of xylem, phloem, lignified vessels, oil globules, cuticles and calcium oxalate crystals under the microscope [14].

Extraction

The course drug powder of each herbal drug sample was accurately weighed and transferred in thimble prepared by filter paper and thimble filled with drug was placed in extraction chamber in Soxhlet apparatus. The extraction was started with non-polar solvent (Petroleum ether) first and collect the extract in round bottle flask. The extract was made dry with help of rotator evaporator.

Phytochemical testing

The phytochemical analysis was performed according to the procedure mentioned in Ayurvedic Pharmacopoeia of India.

Preparation of decoction

All crude drugs were taken in glass vessel and filled with one liter of water and boiled the mixture till $1/4^{th}$ of the liquid was remained. The filtrate of liquid was collected through filtration of mixture. The collected filtrate of decoction was dried at low pressure using rotator evaporator and the dried residue was collected in aired dried container to avoid the moisture. This collected residue of decoction was used to prepared aqueous solution of decoction at different concentrations (1.0, 1.5 and

2.0 percent) used for preparation of herbal extract sugarcane jaggery.

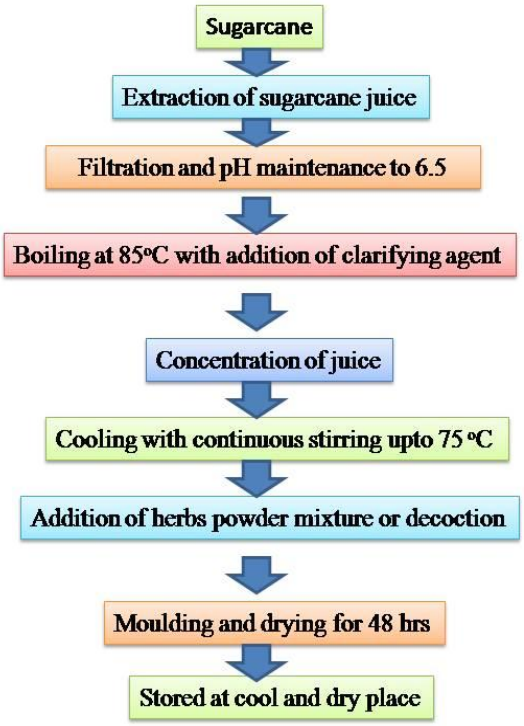
Preparation of medicated jaggery

The herbal drugs were used in preparation of medicated jaggery. The composition of added jaggery contains total six ingredient (Jaggery, Shatavary root, Ashwagandha root, dried ginger rhizome, black pepper fruit and long pepper fruit) in different concentrations listed in table no 1. Total six formulations were prepared in which three formulations contain the decoction of herbal drugs at different concentrations (1%. 1.5% and 2% w/w) and named as Decoction added medicated jaggery (DAMJ-1, DAMJ-2 and DAMJ-3). Other three preparations were made by using the herbal drug powder in different ratio (1%, 1.5% and 2%) and named as powder added medicated jaggery (PAMJ-1, PAMJ-2, and PAMJ-3) [15].

Table 1: Composition of added jaggery

S.N.	COMPONENT	BOTANICAL SOURCE	PART USED	FRACTION
1.	Sugar cane juice	*Sacchrum officinarum*	Stem	Base
2.	Ashwagandha	*Withania somnifera (L.)*	Root	4 parts
3.	Shatavari	*Asparagus resimosus willd.*	Root	3 parts
4.	Dried ginger	*Gingiber officinales*	Rhizome	1 part
5.	Black pepper	*Piper nigrum*	Fruit	1 part
6.	Long pepper	*Piper longum*	Fruit	1 part

Preparation of medicated jaggery



Physical properties of DAMJ AND PAMJ.

The physical properties of both type of medicated jaggery were evaluated on pH, hardness, moisture content, color and insoluble solid.

Nutritional values of DAMJ AND PAMJ per 100 grams

The nutritional value of jaggery and medicated jaggery was performed according to standard protocol. The nutritional value like carbohydrates, mineral, vitamins and fibers were evaluated and listed below in table 3.

Antioxidant properties of DAMJ and PAMJ by DPPH method.

The oxidative stress is the major cause of cellular injury. The various free radicals generated by medicines promote the oxidative damage of cells develop other diseases. The imbalance between the oxidants and antioxidants leads to the oxidative stress. Vitamin E and C are used as antioxidant for scavenging the free radicals. There are various in vitro methods for the evaluation of antioxidant activity of herbal drugs among which the DPPH method is most popular method.

Reagents and Materials: DPPH (2,2-diphenyl-1-picrylhydrazil), Methanol, Prepared test and standard samples in respective solvent, Cuvettes, Pipettes and tips.

Preparation of DPPH solution: The 0.1mM stock solution of DPPH was prepared by dissolving the accurately weighed DPPH for 0.1mM in methanol and stored the prepared stock solution in dark color bottle at cool temperature [16].

Procedure:

- The test tubes were arranged and labeled as control, standard, DAMJ 1, DAMJ 2, DAMJ 3, PAMJ 1, PAMJ 2 and PAMJ 3 of different concentrations in triplicate.
- The control was prepared by addition of 0.5 ml of DMSO and 0.5 ml of DPPH solution.
- 0.5 ml of DPPH solution was added in all test tubes of standard (Ascorbic acid) and test preparations.
- 0.5 ml solution of all preparations was added in all test tubes except control test tube.
- The control test tube contains 0.5 ml of DMSO and 0.5 ml of DPPH solution.
- Ascorbic acid was used as standard.
- Each test tube was incubated at 37°C for 30 min.
- The absorbance of each solution of control, standard and tests was measured at 517 nm in UV-Visible spectrophotometer.
- The percentage of scavenging of all the formulations was calculated by applying the formula given below.

Scavenging DPPH % = [($_{Abs}$Control – $_{Abs}$Sample) / $_{Abs}$Control] x 100

The IC_{50} value of each sample and standard were calculated.

***In vitro* thrombolytic activity of added jaggery formulations**

In vitro thrombolytic activity of different jaggery formulation was carried out according to following method.

- 21 ml of venous blood was collected from slaughter house and transferred to different pre weighed sterilized micro-centrifuge tube (1ml/tube).
- The micro-centrifuged tubes were incubated for blood clotting at 37◦C for 45minutes. After clot formation removed serum completely.
- Each of the tube containing clot was again weighed to determine the weight of the clot.
- The clot weight = weight of clot containing tube — weight of tube alone.
- Each micro-centrifuge tube containing clot was appropriately labeled and 100 µl of the plant extract of different concentration was added to the tubes accordingly.
- As a positive control, 100 µl of streptokinase and as a negative non thrombolytic control, 100 µl of hydroalcoholic solution were added to the specific marked control tubes.
- The tubes containing clot and plant extract were incubated at 37◦C for 90 minutes for observation of clot lysis.
- The experimental tubes were then centrifuged and the supernatant fluid was completely removed and discarded.
- The experimental tubes were again weighed to observe the difference in weight after clot disruption.
- Finally, difference obtained in weight of the experimental tubes was calculated and the result was expressed as percentage of clot lysis by the following equation,

[Percentage of clot lysis =Weight of released clot/Clot weight ×100]

RESULTS

Table 2: Physical evaluation of added jaggery

	Formulation	pH	Hardness (kg/cm^2)	Moisture content (%)	Insoluble solid (%)
1	Jaggery	7.09±0.01	5.12±0.02	6.12±0.03	2.15±5.23
2	DAMJ-1	6.05±0.06	4.09±1.30	6.15±1.23	1.98±3.23
3	DAMJ-2	6.12±1.00	4.12±1.02	5.46±2.33	1.87±2.36
4	DAMJ-3	6.13±0.02	4.06±1.11	5.14±3.21	1.96±0.89
5	PAMJ-1	6.18±0.02	5.00±1.03	5.89±4.23	1.05±2.03
6	PAMJ-2	6.16±0.03	4.91±1.09	5.96±2.01	2.16±1.32
7	PAMJ-3	6.15±0.02	4.90±1.12	5.66±0.15	2.06±5.26

Table 3: Nutritional values of added jaggery

S.N.	Parameters	Sugarcane jaggery (mg)	Different Formulations (Observed Value)					
			DAMJ 1	DAMJ 2	DAMJ 3	PAMJ 1	PAMJ 2	PAMJ 3
	Minerals							
1.	Calcium	40-100	55	52	52	56	45	53
	Potassium	1056	1001	1202	1203	1423	958	1452
	Sodium	19-30	20	19	21	25	28	24
	Chloride	5.3	4.2	3.4	3.6	2.5	5.2	5.3
	Copper	0.1-0.9	0.1	0.2	0.1	0.2	0.1	0.1
	Iron	10-13	20	19	21	22	16	15
	Zinc	0.2-0.4	0.1	0.2	0.2	0.1	0.2	0.2
	Carbohydrates							
2.	Glucose	1.57	1.52	1.42	1.32	0.95	1.51	1.6
	Sucrose	72-78	75	74	65	68	65	56
	Fructose	1-5.7	2.3	1.5	3.6	4.2	5.5	4.2
	Vitamins							
3.	A	3.8	0.2	0.6	1.2	0.5	0.6	0.8
	B1	0.01	0.01	0.02	0.01	0.01	0.02	0.02
	B2	0.06	0.05	0.06	0.02	0.03	0.04	0.05
	B5	0.01	0.01	0.01	0.01	0.01	0.01	0.01
	B6	0.01	0.01	0.01	0.01	0.01	0.01	0.01
	C	7.0	5.0	4.0	4.2	5.1	2.5	4.0
	E	111.3	99.0	102.5	100	102.3	105	98

Table 4: Antioxidant activity IC50 value of added jaggery

S.N.	Formulation	IC50	S.N.	Formulation	IC50
1.	Jaggery	77.66±1.98	1.	DAMJ-3	41.48±3.80*
2.	Ascorbic Acid	90.09±2.83$^{###}$	2.	PAMJ-1	64.13±3.34***
3.	DAMJ-1	55.00±4.12***	3.	PAMJ-2	54.88±3.34**
4.	DAMJ-2	50.00±3.64***	4.	PAMJ-3	56.41±3.51**

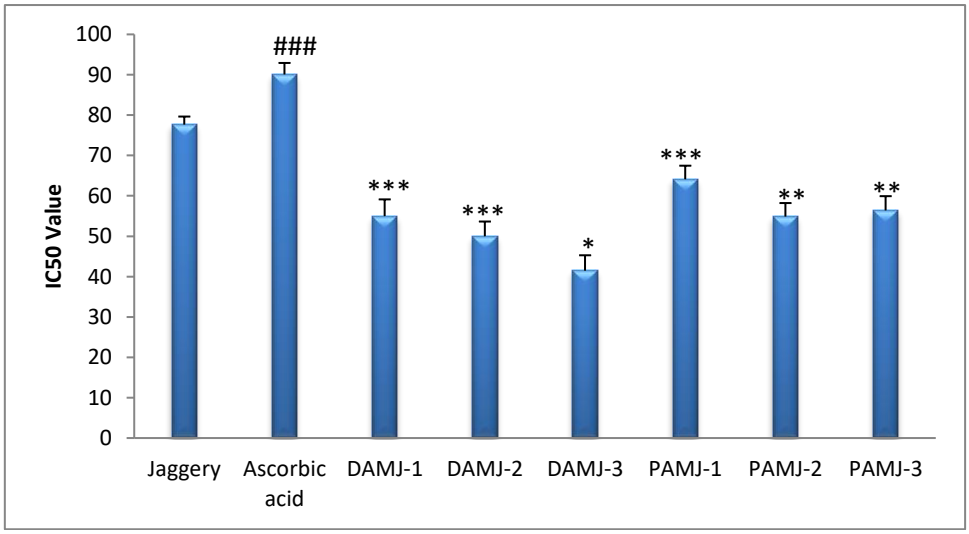


Fig 1: Graph shows the IC50 value of different formulations of added jaggery in DPPH assay

***In-vitro* thrombolytic activity**: The in vitro thrombolytic activity of added jiggery was performed and the result shows at higher concentration the blood clot was dissolved 10.35 ± 2.05 %. The 100 µl streptokinase shows clot lysis 65.36±3.56 % when compared with the blank. The study shows added jiggery contains ashwagandha root, shatavari root, black pepper fruit, long pepper fruit, and rhizome of

dried ginger. Since phytochemical analysis showed that the crude extract contains tannin, flavonoids and phytosterols as common it could be predicted that these phytochemicals may be responsible for its clot lysis activity.

Table 4:

S.N.	Test Tube No	Composition	% blood clot lysis
1.	1	Blood clot + distilled water (100 µl)	02.15 ± 3.15
2.	2	Blood clot + Streptokinase (100 µl)	65.58 ± 4.5$^{###}$
3.	3	Blood clot + DAMJ-1 (100 µl)	38.59 ± 3.56***
4.	4	Blood clot + DAMJ-2 (100 µl)	40.36 ± 5.21**
5.	5	Blood clot + DAMJ-3 (100 µl)	42.25 ± 3.48**
6.	6	Blood clot + PAMJ-1 (100 µl)	25.36 ± 6.21***
7.	7	Blood clot + PAMJ-2 (100 µl)	31.21 ± 5.62**
8.	8	Blood clot + PAMJ-3 (100 µl)	36.48 ± 8.25*

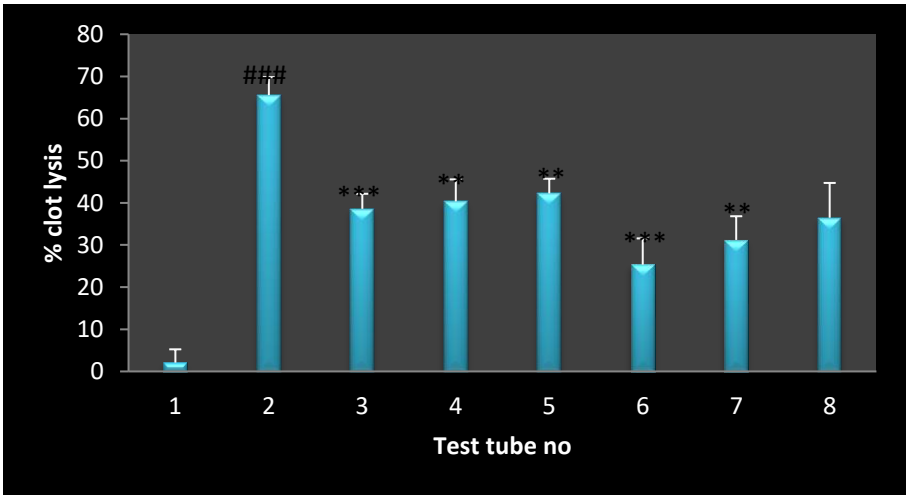


Fig 2: The *in vitro* thrombolytic study on blood clot lysis was performed and found that the added jiggery with decoction of mixture of herbal drugs shows moderate activity of clot lysis in comparison to standard streptokinase. The powder of herbal test drugs shows mild clot lysis activity when compared with standard streptokinase.

DISCUSSION

Sugarcane, a member of the grass family, has the potential to grow up to 4.5 m in height under tropical conditions [17]. It belongs to the genus *Saccharum*, which comprises six species, namely *S. officinarum*, *S. barberi*, *S. sinense*, *S. robustum*, *S. spontaneum*, and *S. edule* [18]. Sugarcane serves as a vital raw material for agro-processing industries producing sugar, jaggery, khandsari, and other industrial products. India is the largest consumer and the second-largest producer of sugar in the world [19].

Black pepper (*Piper nigrum* L.) is a flowering vine belonging to the family Piperaceae and is native to South India. It plays a significant role in India's spice export sector [20]. As one of the oldest known spices, black pepper has a long history of use in traditional medicine. The fruit is a drupe commonly known as a peppercorn [21]. Black pepper extract contains tannins, saponins, flavonoids, essential oils, chavicine, and piperine, which contribute to its medicinal properties [22].

Ginger (*Zingiber officinale*) belongs to the order Zingiberales and is characterized by underground rhizomes [23]. Ginger extracts are rich in bioactive compounds such as gingerols and other secondary metabolites, including saponins, tannins, cardiac glycosides, carbohydrates, and proteins. These constituents are responsible for its anti-inflammatory, antioxidant, antihyperlipidemic, anticancer, antiarrhythmic, anti-obesity, and antidiabetic activities [24].

Piper longum Linn., commonly known as long pepper, belongs to the family Piperaceae and is widely distributed in tropical and subtropical regions. The fruit contains piperine as the principal active phytoconstituent, with a content of about 3–5%. The fruits also contain starch, proteins, volatile oils, alkaloids, saponins, and carbohydrates. Traditionally, *P. longum* is used in treating gastrointestinal,

respiratory, hepatic, and inflammatory disorders, and its roots are used in cardiovascular diseases [25]. Ashwagandha (*Withania somnifera* Linn.) is an important medicinal herb in Ayurveda, used since ancient times. Classical Ayurvedic texts describe its therapeutic properties, while modern studies confirm its adaptogenic and immunomodulatory effects. The stem contains withanolides, and the roots contain alkaloids, β-sitosterol, polyphenols, fatty acids, and phytosterols, which contribute to its wide range of pharmacological activities.

Shatavari (*Asparagus racemosus*) is described in Ayurveda as a *rasayana*, promoting vitality and cellular resistance. It is traditionally used for treating hyperlipidemia, hypertension, dysmenorrhea, urinary disorders, and reproductive health conditions. The roots contain steroids, flavonoids, alkaloids, furan derivatives, and essential oils, contributing to antioxidant, cardioprotective, immunostimulant, and neuroprotective properties [26]

CONCLUSION

The present study revealed the additive property of added sugarcane jaggery. The antioxidant property of added jaggery shows that the decoction added jiggery (DAMJ) was more active than the powder added jaggery (PAMJ). In fibrinolytic activity the decoction added jaggery (DAMJ) was also found more active than the powder added jaggery (PAMJ).

Conflict of interest

There is no conflict of interest between the authors

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