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DEVELOPMENT AND EVALUATION OF ANTI-INFLMMATORY GEL FROM *AMORPHOPHALLUS PAEONIIFOLIUS*

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ABSTRACT

Objectives: Validate the study of phytoconstituents from the extracts of *Amorphophallus paeoniifolius* tubers for the Anti-Inflammatory activity and prepare the extract into gel form and evaluate the gel formulation for anti-inflammatory study. **Methodology:** *Amorphophallus paeoniifolius* tuber extract which was successively extracted in various non-polar to polar solvents. Performed preliminary phytochemical tests for *Amorphophallus paeoniifolius* tuber extract. The extract was evaluated for Flavonoid content, phenolic content and antioxidant activity. HRBC study for Anti- Inflammatory activity for various extract was performed. TLC & comparative TLC for methanolic extract was carried out. Comparative microbial test of *Amorphophallus paeoniifolius* tuber extract with marketed formulation on *S.aureus* stains. Formulations were prepared and evaluated on different parameters. **Results**: *Amorphophallus paeoniifolius* tuber extract showed the maximum presence of secondary metabolites in methanolic extract. The total flavonoid content were found to be 11.13 ± 0.118 and 14.83± 0.455 mg of QE/g in methanol extract and total phenolic content was found to be 8.54 ± 0.043 and 10.26 ± 1.66 mg of GA/g in methanol extract and *Amorphophallus paeoniifolius* tuber extract showed significant antioxidant activity in methanolic extract. Methanolic extract showed the maximum anti-inflammatory activity for HRBC. The comparative microbial study with marketed formulation shows good results and thus herbal formulation of *Amorphophallus paeoniifolius* tuber *may* be used to cure Inflammation. **Conclusion:** From this study, we concluded that *Amorphophallus paeoniifolius* tuber may be used for the inflammation.

Keywords: anti-inflammatory, *Amorphophallus paeoniifolius*, methanolic extract, microbial, HRBC

INTRODUCTION

Inflammation is a tissue's and its microcirculation's response to a pathogenic outcome. The production of inflammatory mediators, as well as the migration of fluid and leukocytes from the bloodstream into extravascular tissues, characterise this condition **[1]**.

The inflammation response's main goal is to remove pathogenic insults and injured tissue components. This procedure either results in the restoration of normal tissue architecture and physiological function, or in the formation of scar tissue to replace what cannot be repaired[7]. Inflammation, however, appears to cause more harm than good in certain circumstances. The cartilage can be destroyed by neutrophil lysosomal enzymes that reach the area when inflammation affects a joint (rheumatoid arthritis, for example). Another example is that tumours appear to form in areas that are chronically irritated. As a result, we refer to inflammation as a two-edged sword **[2]**.

Inflammation has traditionally been classified as acute or chronic, depending on the duration of the damage, clinical symptoms, and the type of the inflammatory response **[1]**. Accumulation of fluid and plasma components in the injured tissue, intravascular activation of platelets **[2]**, and the presence of polymorphonuclear leukocytes are all signs of acute inflammation. Lymphocytes, plasma cells, and macrophages are all characteristics of chronic inflammation **[3]**.

Pathophysiology of Inflammation

Regardless matter the aetiology, the inflammatory response is very similar. Inflammation can be caused by a variety of factors. Inflammation can be caused by a variety of factors, including infection (the presence of living bacteria within the tissue). Inflammation can easily occur even in the most sterile situations, such as when a section of tissue dies due to a lack of blood flow **[2]**.

Acute inflammation is the body's rapid and early response to injury, with the goal of delivering leukocytes to the injury site. Leukocytes arrive and eliminate any invading bacteria before starting to break down necrotic tissues.

There are two essential components to this procedure:

1) Vascular changes: changes in vessel diameter that result in increased blood flow (vasodilation) and structural changes that allow plasma protein to exit circulation (increased vascular permeability).

2) cellular events: leukocyte emigration from the microcirculation and accumulation in the injured site (cellular recruitment and activation) **[4]**

Viral infection, persistent microbial infections, prolonged exposure to

potentially harmful chemicals, and autoimmune illnesses are all examples of chronic inflammation. Chronic inflammation is defined as inflammation that lasts for a long time (weeks, months, or years) and is characterised by active inflammation, tissue injury, and healing all occurring at the same time **[4]**. Aside from inflammation with mononuclear (chronic inflammatory) cells, tissue death, which is predominantly controlled by inflammatory cells, and repair, which involves new vessel growth (angiogenesis) and fibrosis, are also involved **[4]**.

Elephant foot yam (*Amorphophallus paeoniifolius*) is a tropical tuber crop of the Araceae family with a lot of promise. Because of its high output potential and culinary qualities, it is a major tuber crop in tropical and subtropical nations. These tuber have a good anti-inflammatory activity and it is used to treat arthritis, elephantiasis, glandular swelling and others. This plant's tuber has a great therapeutic value and is eaten as a meal. It's linked to acridity (an itching sensation in the mouth and throat) and the presence of elevated oxalates raphides **[1]**. It also contains a lot of vitamin B-6, which helps ladies with premenstrual syndrome. It's a natural product with a lot of fibre. It has a high concentration of vital minerals and can be used as a slimming food because it lowers cholesterol and helps weight loss. People who have traditionally relied on starch-rich diets may be unaware of the nutritional value of new high-yielding elephant foot yam types **[6]**.

MATERIALS AND METHODS

The various material and methods used in research are given below

Amorphophallus paeoniifolius tuber from local market, Chandrapur, Petroleum ether, Ethyl acetate, Ethanol, Methanol, Folin ciocalteu reagent obtained from LOBA chemie, Mumbai. India, Gallic acid from SunCHEM and DPPH from Sisco research laboratory Mumbai. India.

COLLECTION AND AUTHENTICATION:

Dried *Amorphophallus paeoniifolius* tuber were collected from local market of Chandrapur region, Maharashtra and authenticated from botanical department RTM Nagpur University India.

PREPARATION OF EXTRACT: [7]

The continuous hot extraction process was used for the extraction. *Amorphophallus paoniifolius* tubers were cleaned and dried. Approximately 50 gm of *Amorphophallus paoniifolius* tubers were taken into a thimble and placed in a soxhlet apparatus. It was extracted successively by using solvents petroleum ether, chloroform, ethanol, methanol and macerated by hydroalcholic solvents (Methanol: water) and lastly with water.

PRELIMINARY PHYTOCHEMICAL SCREENING: [8]

The methods used for detection of various phytochemicals were followed by qualitative chemical test to give general idea regarding the nature of constituents present in crude drug. The qualitative chemical tests for various phytoconstituents were carried out for all the extracts of *Amorphophallus paeoniifolius* tuber.

TLC FINGERPRINTING: [9]

Thin layer chromatography (TLC) is a chromatography technique used to separate mixtures. To conform the secondary metabolite in the extracts by using silica gel-G as a stationary phase for separation of phytochemical compounds. Extract were spotted to prepared plate manually by using capillary and put into suitable mobile phase. Because of different analytes ascend the TLC plate at different rates, separation is achieved. The petroleum ether and methanol extracts of the *Amorphophallus paeoniifolius* were subjected to thin layer chromatographic analysis, to find the presence of number of chemical constituents to support the chemical test.

DETERMINATION OF TOTAL PHENOLIC CONTENT: [10]

Total phenol content of methanolic extracts of *Amorphophallus paeoniifolius* tuber was determined by using modified Folin-Ciocalteau method. Absorbance of the test sample was measured at λmax = 765 nm. Total phenolic content was expressed as (mg of GAE/g of gallic acid) equivalent using the following linear regression equation based on the calibration curve: (r2 = 0.9989), y = 0.0056x + 0.0103, where x stand for absorbance and y stand for gallic acid equivalent (mg/g).

DETERMINATION OF TOTAL FLAVONOIDS CONTENT: [11]

The total flavonoid content of methanolic extract of *Amorphophallus paeoniifolius* tuber was determined by the aluminium chloride colorimetric method. Absorbance was measured at 510 nm and yellow colour indicated the presence of flavonoids. Total flavonoid content was calculated as quercetin (mg of QE/g) using the following equation based on the calibration curve: (r2 = 0.992), y = 0.0065x + 0.0337 where x stand for absorbance and y stand for quercetin equivalent (mg/g).

ANTIOXIDANT ACTIVITY (DPPH RADICAL SCAVENGING ASSAY): [12]

The total flavonoid content of methanolic extract of *Amorphophallus paeoniifolius* tuber was determined by the aluminium chloride colorimetric method. Absorbance was measured at 510 nm and yellow colour indicated the presence of flavonoids. Total flavonoid content was calculated as quercetin (mg of QE/g) using the following equation based on the calibration curve: (r2 = 0.992), y = 0.0065x + 0.0337 where x stand for absorbance and y stand for quercetin equivalent (mg/g).

IN-VITRO ANTI-INFLAMMATORY ACTIVITY: [13]

In-vitro anti-inflammatory activity of extracts of B. ceiba was assessed by Human Red Blood Corpuscles (HRBC) membrane stabilizing method with slight modifications. The blood was collected from healthy human volunteer who had not taken any antiinflammatory drugs for 2 weeks prior to the experiment and transferred to the heparinized centrifuge tubes and centrifuged at 3,000 rpm. The packed cells were washed with isosaline and a 10% suspension in normal saline was made. Diclofenac potassium (50µg/ml) was used as standard. The reaction mixture (4-5 ml) consisted 2 ml of hypotonic saline (0.25% w/v NaCl), 1 ml of 0.15 M phosphate buffer (pH 7.4), and 1 ml of test solution (1000 µg/ml) in normal saline and 0.5 ml of 10% HRBC in normal saline. For control, 1 ml of isotonic saline was used instead of test solution. The mixtures were incubated at 56°Cfor 30 min. and cooled at running tap water, centrifuge at 3000 rpm for 20 min. The absorbance of supernatant was read at 560 nm using visible Spectrophotometer. The experiment was performed in triplicates. The Percentage membrane stabilization was calculated using the following formula:

$$\% \text{ Inhibition of Haemolysis} = \frac{[\text{Absorbance of control} - \text{Absorbance of test}] \times 100}{\text{Absorbance of control}}$$

FORMULATION OF GEL: [14]

Required quantity of polymer (carbapol-971) was weighed individually, and sufficient amount of distilled water were mixed in a separate beaker, after which it was continuously stirred by mechanical stirrer till the polymer is soaked in the water and kept for 24 h at room temperature. With continuous stirring, now the appropriate quantity of methyl paraben and propyl paraben was added which acts as a preservative. Small quantities of triethanolamine were added with continuous stirring to achieve neutral pH. Finally extract was added to gel with continuous stirring till drug get dispersed completely. The prepared gel was filled and sealed in the aluminium collapsible tube. A similar procedure was followed for base control gel without the extract.

EVALUATION OF GEL FORMUL-ATIONS: [15-17]

Prepared formulations were evaluated for various physicochemical parameters such as colour, homogeneity, pH, spreadability, viscosity and drug content (total flavanoid content).

ACCELERATED STABILITY STUDY: [14]

Stability studies for this present work was carried out at 40ºC ±2 ºC 75% RH for the selected formulation for three months. The

selected formulations were packed in amber bottle. They were stored at 40ºC±2ºC/75±5%RH for 3 months in humidity chamber and evaluated for their physical appearance and various parameters at specified intervals of time.

COMPARATIVE MICROBIAL ASSAY: [18]

Agar well diffusion method: This method is commonly used to check the antimicrobial activity of plants or microbial extracts. Similarly to the procedure utilized in disk diffusion method, the agar plate surface is inoculated by spreading a volume of the microbial inoculum over all agar surfaces. Then, a hole with measurement across object of 6 to 8 mm is punched aseptically with a sterile plug borer or a tip and a volume of standard drug (diclofenac60mg/ml), methanolic extract of (10mg/ml) and formulation optimize batch G7 (1%w/v) introduced in to the wall. Then agar plates are incubated for 24hr, under applicable conditions depending upon the microorganisms.

RESULTS AND DISCUSSION

Authentication of Plant: The plant was authenticated from Botany Department of RTMNU and having the authentication Voucher specimen no. is 10450.

The Preliminary phytochemical constituents are showed in **Table 1**.

THIN LAYER CHROMATOGRAPHY:

Assessment of secondary metabolites were observed by TLC and was confirmed by comparative TLC and the Rf value was match with standard and showed in **Table 2**.

TOTAL PHENOLIC CONTENT:

The phenolic content was calculated using the linear regression equation obtained from standard gallic acid graph (r2 = 0.9989), y = 0.0056x + 0.0103. Among test extracts at concentrations 50µg/ml and 100µg/ml, the total phenolic content was found to be 15.9 ± 0.613 and 12.5 ± 0.412 mg of GA/g in methanol extract of *Amorphophallus paeoniifolius* tuber.

TOTAL FLAVONOID CONTENT:

Flavonoid content was calculated using the linear regression equation obtained from standard quercetin graph (r2 = 0.992), y = 0.0065x + 0.0337. Among test extracts at concentrations 50µg/ml and 100µg/ml, the total flavonoid content were found to 7.47± 0.042 and 9.58 ± 0.056 mg of QE/g in methanol extract of *Amorphophallus paeoniifolius* tuber.

ANTIOXIDANT ACTIVITY STUDY:

Evaluation of scavenging activity on DPPH radicals:

% scavenging of DPPH = (Acontrol – Atest) / Acontrol × 100

The methanolic extract of *Amorphophallus paeoniifolius* tuber showed significant antioxidant activity as compared with the ascorbic acid Shows in **Table 3**. The antioxidant activity of scavenging activity

on DPPH radicals may due to the presence of flavonoids, tannins and phenol.

DETERMINATION OF ANTI-INFLAMMATORY ACTIVITY (HRBC)

The **Table 4** showed the HRBC parameters and its % inhibition by the extract was showed.

EVALUATION OF GEL

The gel formulations of methanolic extract of *Amorphophallus paeoniifolius* batch F7 shows satisfactory result in parameters such as colour and appearance, pH, viscosity, extrudability, spreadability, drug content. Therefore, formulation F7was selected for post stability evaluation and that data was showed in **Table 5**.

COMPARATIVE MICROBIAL ASSAY:

The comparative microbial assay showed that extract may have antimicrobial activity which was showed in **Table 6**.

ACCELERATED STABILITY STUDY:

The accelerated stability was performed for 3 months due to time constraint. Study's parameter and results were showed in **Table 7** for Colour and appearance, pH Viscosity, % Drug remaining.

Table 1: Preliminary phytochemical screening

Sr. No.	constituents	PEE	CE	EE	ME	HAE
1.	Sterols	+	-	+	+	-
2.	Alkaloids	-	+	-	+	-
3.	flavonoids	-	-	+	+	+
5.	Proteins	-	-	-	-	+
7.	Glycosides	-	-	-	-	-

PEE-Petroleum ether extract, CE-Chloroform extract, EE-ethanol extract, ME- methanol extract, HAE-hydroalcholic extract; + Presence, - Absence

Table 2: TLC and comparative TLC of extract

Sr. no.	*Amorphophallus paeoniifolius* tuber	Samples Developing solvents and visualizing agents	Rf values	TLC plate
1.	Methanolic Extract	n-hexane: Ethyl acetate: Formic acid: methanol of the ratio (3:4:3:0.1) Visualizing agents: iodine chamber	0.5	
Comparative TLC with standard				

2.	**Sample (T)-Methanol Standard (S)-quercetin**	**Chloroform: Ethyl aceted (2.5:2.5) visualizing agents: AlCl3 Observed in UV chamber at 360nm**	**T1- 038 T2- 0.45**	

Table No 3: Effects of test extracts on % DPPH inhibition

Conc. (µg/ml)	% scavenging activity on DPPH radicals	
	Ascorbic acid	ME
25	33.12±0.62	18.87±0.81
50	45.38±0.65	29.12±0.72
75	69.98±0.68	38.31±0.59
100	81.66±1.02	45.39±0.68
125	98.27±0.63	56.21±1.02

Values expressed as Mean ± SD, n=3

Table 4 Anti-inflammatory activity of various Extract

Parameters	Absorbance	% inhibition
Control	0.460	-
Petroleum extract	0.358	22.36
Methanol extract	0.335	27.17
Ethanol extract	0.385	16.30
Diclofenc	0.196	57.39

Table 5: Colour and appearance, pH, viscosity, Extrudability, Spreadability, Drug content (phenolic content)

Formulations	Colour and appearance	pH	Viscosity cps	Extrudability	Spredability	Drug content %
G1	Light brown	6.4	614100	81.25±5.6	13.11	24.14
G2	Light brown	6.5	672000	83.27±5.3	13.32	25.64
G3	Light brown	6.8	674000	93.88±3.1	17.20	31.87
G4	Light brown	7.1	685000	90.11±4.6	19.21	39.24
G5	Light brown	6.6	697000	95.56±4.3	21.48	47.54
G6	Light brown	6.9	753000	87.98±2.5	22.24	55.60
G7	Light brown	7	784000	97.45±4.1	25.14	55.90
G8	Light brown	7.1	788000	87.26±5.4	25.10	57.15
G9	Light brown	6.5	791000	98.13±2.6	23.24	57.89

Table 6: Comparative microbial assay in *S. aureus* cultures

Culture	Samples			
S.aureus	Zone of inhibition	Standard	Methanolic extract	Formulation
		3cm	3.2cm	2.8cm

Standard **Methanolic extract** **Formulation**

Table 7: Accelerated stability study of optimise batch F7

Parameter	Storage (in month)			
	Initial	1st month	2nd month	3rd month
Colour and appearance	Light brown	Light brown	Light brown	Light brown
pH	7	7.1	6.9	7.1
Viscosity	784000	77900	78500	771000
% Drug content	55.90	55.65	55.90	55.91

SUMMARY

The main objectives of these study were to formulate evaluate the anti-inflammatory activity of *Amorphophallus paeoniifolius* tuber extract. The successive extraction was carried out in various solvents (non-polar to polar solvents). The yield was calculated in percentage %. The % yield of Petroleum Ether extract was found 1.05%, Chloroform extract was found 1.8%, Ethanol extract was found 4%, Methanol extract was found 3.5%, Hydro-alcoholic extract was found 4.5%. The preliminary phytochemical screening showed various secondary metabolites. TLC fingerprinting showed the presence of various secondary metabolites in *Amorphophallus paeoniifolius* tuber extract. Methanolic extract showed presence of phenol content was found to be 15.9 ± 0.613 and 12.5 ± 0.412 mg of GA/g and flavonoid content was found to be 7.47± 0.042 and 9.58 ± 0.056 of QE/g. The flavonoid and phenolic content was found in satisfactory range when it's compared with previous research paper which I have referred for this study.

The anti-oxidant study shows that scavenging activity on DPPH radicals was found to be higher inhibition of DDPH was exhibited by methanol extract 56.21±1.02 was compared to ascorbic acid. From the HRBC study the maximum Anti-inflammatory activity showed in methanolic extract and it was found to be 57.39 %.

The gel formulation from *Amorphophallus paeoniifolius* tuber extract were showed in good result in various parameter. And the various evaluation parameter was carried out for 9 batches (G1-G9). Among the 9 batches , G7 batch showed the good result and there colour and appearance, pH, viscosity, extrudability, spreadability, and drug content was found to be light brown,7, 784000 cps, 97.45±4.1, 25.41, 55.90% respectively and that's why these batch were selected for the stability study. The results of stability study of the final gel reveal that no changes were noticed in all the tested physicochemical parameter during 3 month in at 40ºC ±2 ºC 75% RH**.** Comparative microbial assay by well diffusion method shows satisfactory results in *E coli* bacteria. The methanolic extracts and formulation inhibits the growth of *E.coli*. Methanolic extracts of

Amorphophallus paeoniifolius tuber exhibits high quantity of flavonoid like quercetin and they shows anti-inflammatory activity as well as the phytoconstituents like phenol, sterols, alkaloids.

CONCLUSION

Methanol extracts *Amorphophallus paeoniifolius* tuber showed the presence of flavonoid content and phenolic content. In-vitro Anti-Inflammatory activity (HRBC) of *Amorphophallu spaeoniifolius* extract showed that it may be beneficial for various inflammatory disease. Flavonoid compounds can act on COX pathway as a NSAIDs so Gel of methanolic extract of *Amorphophallus paeoniifolius* tubers can acts as Anti-Inflammaory gel.

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