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Evaluation of Analgesic Property of *Drynaria quercifolia* Rhizome Extract

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Authors' contributions

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

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ABSTRACT

Analgesics are drugs that primarily relieve pain. The main class of painkillers is narcotics, which include additional drugs that are chemically based on morphine molecules. Phytochemicals are a more recent evolution of the term that highlights most of the plant sources of these protective and preventive compounds. Many herbal preparations are prescribed as analgesics in the traditional literature. The search for new analgesics from a large number of medicinal plant resources is intensifying. In this study, we performed a phytochemical screening and analgesic activity of the bark of *Drynaria quercifolia*. Phytochemicals have shown that the presence of terpenoids in flavonoids, phenols, saponins, glycosides, alkaloids, polyphenols, proteins, triterpenoids, bark of Drynaria quercifolia has been proven as analgesic activity. *Drynaria quercifolia* has strong analgesic properties mediated by peripheral and central inhibitory mechanisms. The potential mechanism of action of *Drynaria quercifolia* may be due to the synergistic action of the phytochemicals present in it. This can provide a justification for the use of this plant in pain and inflammatory diseases in natural medicine.

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1. INTRODUCTION

"Pain is well-defined by the IASP (International Association for the Study of Pain) as an disagreeable sensual and emotional experience related with actual or potential tissue damage, or has been defined with regard to such an injury" [1]. Individuals can describe their pain in terms of severity, duration, and type. Since the emotional response of a patient's syndrome to a painful stimulus contributes greatly to the experience of pain, the patient's, reports of the severity of pain caused by seemingly similar stimuli often show significant differences between individuals. Therefore, there is no objective measure that can be used effectively to compare the severity of pain in one person's syndrome with pain in another. Instead, many pain measures have been designed and validated to quantify changes in pain severity in individuals[2]. "Pain is certainly an unpleasant sensation, but overall it is usually beneficial for humans and animals.This is mainly a protective mechanism of the body, which is not only a pain but also a pain, which occurs whenever tissues are damaged, causing the individual to react and eliminate painful stimuli"[3]. "Usually, this is a direct response to adverse events associated with tissue damage, such as injury, inflammation or cancer, or, but can cause severe pain regardless of the apparent predisposing cause (for example. Trigeminal neuralgia), or a precipitating injury that continues long after it has healed (such as phantom limb pain). It can also occur as a result of brain or nerve damage (such as after a stroke or herpes infection). In many pathological conditions, tissue damage is a direct cause of pain, and these lead to the local release of various chemicals, including, which are thought to act on nerve endings, either directly activated, or increase sensitivity to other forms of stimulation" [4]. "The choice of a suitable analgesic is based on taking into account the risk factor for each class of drug according to the type of pain, which is, the severity of the pain and the risk of side effects. Pain has traditionally been classified into two classes, acute and chronic, but the severity and expected survival of patients are other factors that need to be considered in drug selection"[5].

Although many synthetic drugs of today's syndrome originate from the plant kingdom, herbalism has historically declined when herbalism has established itself as a leading and effective area of medical treatment. Plants are a gift from nature's to humans, to make a healthy life without disease. It plays an important role in maintaining our health. India is one of the most culturally diverse countries in the world where the medicinal plant sector is part of a venerable tradition that is still respected today. Traditional drug derives its scientific inheritance from the rich knowledge of ancient peoples. Therefore, it is not astonishing that the rights of traditional medicines apply to some "difficult diseases that are " refractory[6]. India is well known for its traditional health care system, namely the health care system. In addition to Siddha, Ayurveda, Unani and Amchi (Tibetan), there are also vast reservoirs of the living traditions of ethnomedicine. The first reference to the use of plants in medicine is in Rigveda, written between 4500 and 1600 BC. In the British era of western culture, our traditional art of natural healing is gone. Now it appears again, because it recognizes the importance of curing the disease without side effects. *Drynaria quercifolia* J, known locally as Guar. Smith is a parasitic fern widespread in Bangaladesh, India and Thailand. Traditionally, it has been reported that the leaves of the plant have been used by the tribal communities of Tamil Nadu and Kerala to treat various diseases such as typhoid, typhoid, typhoid, typhoid, typhoid, and other diseases, chronic jaundice and anti-inflammatory agents, such as compresses and fertility drugs, and as antipyretic agents. The whole plant is used to treat diseases of the chest and skin, and is also an anthelmintic, expectorant and tonic. The aim of this study is to evaluate the phytochemical and analgesic activity of methanol extract from the rhizome of *Drynaria quercifolia*.

2. MATERIALS AND METHODS

2.1 Chemicals and Animals

Sodium hydroxide, acetic acid, formalin, trichloroacetic acid (TCA) and aspirin were purchased for the Sigma Chemical Company in Mumbai. All other chemicals and reagents used in the study were obtained from Glaxo and Cisco Laboratories in Mumbai, India, with high-purity analytical quality. The study used male albinolates of the Wister strain weighing about 180 to 190 grams. All the animals were given a standard pellet diet and the water was given *ad*

libitum. We adapted to the environment for a week before using it experimentally. The experiments were conducted in accordance with the guidelines of the Animal Experimental Management Supervision Committee ((Ethical No: CPCSEA/265), New Delhi, India.

2.2 Collection of Plant Materials

The rhizomes of *Drynaria quercifolia* (Tamil Name: Aatukaal Kilangu) were collected from Kolli Hills, Namakal District, Tamil Nadu.

2.3 Preparation of Methanolic Extract

Drynaria quercifolia powder was soaked for 3 days with 70% methanol at room temperature. After 3 days, I transferred the food to Chinese cuisine. Supernatant put a Chinese dish on a 45-degree boiling water bath and completely removed it. After complete elimination of the alcohol, a semi-solid extract is obtained. The resulting residues were kept in the refrigerator for later use. The extract was composed up to a known volume in distilled water shortly before oral administration.

2.4 Phytochemical Screening

Using standard procedures to identify the components described by Sofowara, [7], chemical tests were performed on methanol extracts and powder samples; Trease and Evans, [7,8] and Harborne, [9].

2.5 Pain-relieving Activity

The analgesic activity of the methanol extract in the rhizomes of *Drynaria quercifolia* was analyzed in various ways by acetic acid induced formalin test, writhing assay, and tail immersion method.

2.6 Experimental Design

Group I: Animals derived from certain substances for this study

Group II: The animals in this group were given plant extracts with a weight of 100 mg/kg. Group III: This group of animals received a plant extract at a dose of 250 mg/kg of weight. Group IV: The animals in this group received plant extracts at a dose of 500 mg/kg. Group V: The animals in this group received a standard aspirin drug at a weight of 150 mg/kg. After the last administration of the plant extract and the norm, determine the analgesic properties after the prescribed period.

2.7 Analegsic Activity

2.7.1 Acetic acid-induced milking method

The animals were divided into five groups, each containing six animals.The first group received saline solution, while the second, was treated with saline, third and fourth groups received 100,250 and 500 mg/kg of extract, respectively, and the fifth group received 150 mg/kg of aspirin/kg. After 60 minutes, 0.6% acetic acid (10 ml/kg) (ip) is injected. The number of abdominal contractions for the next 15 minutes was counted. The reduction in the number of tortillons compared to the control group was considered as evidence of the presence of analgesia [10]. Each animal was marked and monitored for identification.

2.7.2 Formalin test

The method used was similar to the method described by Shibata et al. [11]. 20 ml of 1% formalin were injected subcutaneously into the right rear foot of the mouse. The time spent on the reaction of licking or biting the injected foot was taken as an indicator of the reaction of pain. The reaction of 15– 30 min was measured after injection of formalin (phase 1) for 5 minutes and after injection of formalin (phase 2). Extract(150mg/kg BW. And 300 mg/kg bw) and indomethacin (10 mg/kg bw) were administered for 45 minutes prior to formalin injection. The pilot animal received the car. Each animal was marked and monitored for identification.

2.7.3 Tail immersion test

The rats were divided into six groups, each containing five animals. The 5 cm lower part of the tail was soaked in water beakers held at 55±0.5°C [12]. The second water tail removal time was taken as reaction time, and the quenching cut-off time was set to 10 seconds. The reaction time was measured for one hour after oral administration of EtOH and water extracts (100 250 and 500 mg/kg). Morphine (10 mg/kg) was administered subcutaneously one hour before the test. Each animal was marked and monitored for identification.

2.8 Statistical Analysis

The value is represented as an average ± SD for 6 rats. The data were analyzed by a unidirectional ANOVA followed by a post-hoc HSD Tukey test using SPSS ver. 20. Statistically significant variation was obtained by comparing Group I with Group II, Group III, Group IV and

Group V. *P<0.05 is statistically significant compared to group I (normal) and NS= not significant (P>0.05).

3. RESULTS AND DISCUSSION

The rhizomes of *Drynaria quercifolia* used acetic acid-induced weighting, formalin testing, and tail immersion methods to evaluate phytochemicals and analgesic activity. This study was conducted for the phytochemical analysis and analgesic activity of methanol extract from the rhizome of *Drynaria quercifolia*. Different concentrations of methanol extract from the rhizome of *Drynaria quercifolia* (100 250 and 500 mg) were used for analgesic activity. The results are presented in the Table 1 shows a phytochemical analysis of the rhizome extracts of *Drynaria quercifolia* The results show the presence of flavonoids, phenols, saponins, alkaloids, glycosides, polyphenols, proteins, triterpenoids and terpenoids. The Tannins and the phlobatanninsdid not exist.

Table 1. Phytochemical screening of *Drynaria quercifolia* rhizome

S. No	Phytochemical	Result
1	Tannin	-
2	Phlobatanins	-
3	Saponin	+
4	Flavonoid	+
5	Steroid	-
6	Terpenoids	+
7	Triterpinoid	+
8	Alkaloid	+
9	Carbohydrate	+
10	Polyphenol	+
11	Anthroquinone	+
12	Protein	+

(+) Presence (-) Absence

3.1 Acetic acid Writhing Response

The result of the angelic response induced by acetic acid is that the rats exhibit analgesic activity when the methanolic extract of *Drynaria quercifolia* is presented in Table 2, 100, 250 and 500 mg/kg. The dose of *Drynaria quercifolia* is not indicated in the mandate compared to the control group. This results in a significant decrease. Aspirin (150 mg/kg bw), used as a standard medicinal product, had a significant analgesic effect on all observation times compared to control values. Among the different doses, 500 mg/kg potentially reduced the desire induced by acetic acid and the inhibition rate was 66. 17.

Table 2. Effect of *Drynaria quercifolia* on acetic acid induced writhes in rats

Groups	No. of writhes	% of inhibition
Group I	204±5.55	-
Group II	152± 3.60*	25.49
Group III	109±2.98*	46.56
Group IV	69±2.15*	66.17
Group V	75 ±1.96*	63.23

*The value is represented as an average ± SD for 6 rats. Statistically significant variation was obtained by comparing Group I with Group II, Group III, Group IV and Group V. *P<0.05 is statistically significant compared to group I (normal) and NS= not significant (P>0.05)*

3.2 Formalin Test

In rats, significant dose-dependent inhibition of both phases of the formalin-induced pain response was observed (Table 3), with a stronger effect on the second stage than the first stage. Paracetamol (150 mg/kg bw) also significantly inhibited similar methods compared to the control group. Among the different doses, 500 mg/kg significantly reduced the response to pain in both phases. In the second stage of 500 mg/kg of plant extract, the degree of inhibition was from 75.84 to 82.08.

Table 3. Effect of *Drynaria quercifolia* on formalin test in rats

Groups	Pain 0-5 (mins)	% of inhibition	Pain 15-30 (mins)	% of inhibition
Group I	67.50±8.03	-	178 ± 5.36	-
Group II	33.00±5.36*	51.49	120 ± 3.41*	32.58
Group III	15.10±4.16*	77.61	97 ± 3.01*	45.50
Group IV	12.50±4.08*	82.08	43± 2.53*	75.84
Group V	24.83±3.91*	63.23	124 ± 3.57*	30.33

*The value is represented as an average ± SD for 6 rats. Statistically significant variation was obtained by comparing Group I with Group II, Group III, Group IV and Group V. *P<0.05 is statistically significant compared to group I (normal) and NS= not significant (P>0.05)*

Table 4. Effect of *Drynaria quercifolia* on tail- immersion test in rats

Groups	Reaction time (s)	Inhibition (%)
Group I	7.56±2.25	-
Group II	4.85±0.97*	35.84
Group III	3.52±1.28*	53.43
Group IV	3.15±1.25*	58.33
Group V	3.38±0.59*	55.29

*The value is represented as an average ± SD for 6 rats. Statistically significant variation was obtained by comparing Group I with Group II, Group III, Group IV and Group V. *P<0.05 is statistically significant compared to group I (normal) and NS= not significant (P>0.05).*

3.3 Tail-immersion Test

The result of methanolic extract on tail-immersion tests are represent in Table 4. The extract (100, 250, and 500mg/kg) produced dose dependent inhibition of pain. Among the different doses, 500mg/kg has reduced the ache (58.35 %) than other doses. The standard drug morphin also reduces the pain (59.29 %).

"Abdominal whining induced by acetic acid is a sensitive method for evaluating analgesics with peripheral action. The abdominal contractile response induced by acetic acid is a sensitive procedure for evaluating analgesics with peripheral action" [13]. "In general, acetic acid causes pain and stimulates nerve endings by releasing endogenous substances such as serotonin, histamine, prostaglandin (PG), bradykinin and substance P. Local peritoneal receptors have been postulated to be involved in the abdominal contractile reaction" [14]. This method is also associated with increased levels of PGE2 and PGF2 in prostanoids in general, or peritoneal fluid [15], and with lipoxygenase products [16].

The significant reduction in acetic acid-induced eels by *Drynaria quercifolia* suggests that analgesic effects may be mediated at the periphery by inhibition of synthesis and release of PG and other endogenous substances. Several tests (acute and chronic) were used to evaluate the analgesic effects of methanolic extracts of *Drynaria quercifolia.* Different tests should be applied with regard to the quality, intensity and duration of the stimulation, so that the analgesic properties of the substance are as well understood as possible with the help of behavioral nociceptive tests [17]. The results obtained indicate that the extract has the highest dose-dependent analgesic effect for acetic acid induction stimulation tests than the standard drug.

"Recent studies have shown that the initial stages of formalin-induced pain directly reflect the formalin of nociceptors, while later stages reflect that inflammatory pain appears to be attributable to prostaglandin synthesis" [11,18]. "Our results showed a significant inhibitory effect of Drynaria quercifolia rhizome extract on the late-stage nociceptive response of inflammatory pain models in formalin trials. A formalin test may be a more useful model of clinical pain in which later stages depended on peripheral inflammation and changes in central treatment" [18]. "Histamine, serotonin, prostaglandin, nitric oxide, and bradykinin are involved in the later stages of formalin testing" [18]. There is strong evidence that peripheral inflammatory procedures are involved in the later stages. The inhibitory effect of the methanolic extract of the nociceptive reaction of the rhizome of Drynaria quercifolia in the later stages of formaldehyde testing suggests that the anti-nociceptive effect of the rhizome of Drynaria quercifolia can be attributed to its peripheral action.

In this study, the extract had a significant effect in the tail infiltration test at different concentrations. "Maximum inhibition was observed in 500 mg of plant extract (64.16%). A significant increase in the pain threshold produced by *Drynaria quercifolia* in these models suggests the involvement of central pain pathways. Pain is intensely regulated by a number of complex processes, including opiates, dopaminergic, descending noradrenergic and serotoninergic systems" [19,20,21]. The analgesic effect produced by plant extracts of methanol may be by the central mechanisms containing these receptor systems or by peripheral mechanisms involved in the inhibition of prostaglandins, leukotrienes and other endogenous substances that are important players in inflammation and pain, are also known to be. Intensive painkillers increase the pain threshold in animals at both heat and pressure. The effect of the extract on

this pain model indicates that the extract can act centrally.

4. CONCLUSION

The results obtained in this study show that *Drynaria quercifolia* has strong analgesic properties mediated by peripheral and central inhibitory mechanisms. The potential mechanism of action of *Drynaria quercifolia* may be due to the synergistic action of the phytochemicals present in it. This can provide a justification for the use of this plant in pain and inflammatory diseases in traditional medicine.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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