Manufacture of Cold and Hot Chemical Packs and Anti-Inflammatory Potential of *M. Oleifera* Seed Oil as an Alternative Method of Treating Minor Sport Injuries.

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Abstract

Chemical intake has become a leading problem of the 21st Century. This has led to challenges like drug reactions, misuse, abuse, overdose and addiction. Thus, there is a need to seek other alternatives of treating pain. Phytochemical analysis was carried out on *Moringa oleifera* seeds and the following were found to be present; flavone aglycones, saponins, alkaloids, terpenoid, steroids, and tannins. The isothiocyanates present in the *Moringa oleifera* seeds have been shown to be responsible for its anti-inflammatory property (Mahajan *et al*., 2007). Thereafter, physicochemical analysis was carried out on the oil sample which was extracted by cold press method. The acid value, peroxide value, iodine value and refractive index of the oil were found to be (1.23±0.02)mgKOH/g, (10.80±0.12)mEqO_2/g, (67.87±0.54)I_2/100g and (1.4668±0.03) respectively. The oil was topically applied to the site of injury of six subjects who suffered from muscle strain and tear to create a moist surface. Cold packs made from KCl and NH_4Cl were placed on the site of injury on day one, followed by hot packs made from Anhydrous CH_3COONa and $CaCl_2.6H_2O$ in other to alter deep tissue temperature, reduce inflammation and facilitate healing. Subjects were recorded to have recovered by day four of treatment.

Keywords: phytochemical, inflammation, topically, cold press, chemical

Introduction

The oldest form of thermotherapy recorded in ancient times includes; heated sand, rocks, water, animal hides and plant fibers. Such treatments were described as early as some of the Egyptian papyrus writings. (Rehak, 2014). However, thermotherapy has been improved upon to include electrical, chemical, microwave, and so on.

Chemical Packs

Chemical heating pads work based on exothermic or endothermic chemical reactions; as a result, it could either be a hot pad or a cold pad depending on the type of reaction that takes place in the pack. If the reaction is endothermic, then it is a good way of making a cold pad because in this process, heat is absorbed from the environment. On the other hand, when the reaction is exothermic, it is a good way of making hot pack because it liberates heat to the environment.

An example of a Hot pad equation

$CH_3COONa \longrightarrow CH_3COO^- + Na^+$ Exothermic

An example of a Cold pad equation

$NH_4Cl \longrightarrow NH_4^+ + Cl^-$ Endothermic

Heat packs are generally used for outdoor recreational activities such as hiking, fishing and so on. Cold packs are also used for keeping first aid box and food or drinks cold. Hot packs could also be used for maintaining the temperature of sensitive compounds and storing camera batteries in cold regions.

Thermotherapy

Thermotherapy consists of the application of heat or cold (Cryotherapy) for the purpose of changing the cutaneous, intra-articular and core temperature of soft tissue with the intention of improving the

symptoms of certain conditions(Hurley *et al*., 2008).It has been used for centuries to treat backaches, headaches, migraine, arthritis, muscle and joint pain.

Therapeutic effect of heat

These includes; increasing the extensibility of collagen tissues, decreasing joint stiffness, reducing pain, relieving muscle spasm, reducing inflammation, edema and aids in the post-acute phase of healing, and increasing blood flow. The increased blood flow to the affected area provides proteins, nutrients and oxygen for better healing (Prentice, 2008) which is enhanced by the anti-inflammatory properties of essential oil. It can also be beneficial to those with arthritis and stiff muscles and injuries to the deep tissues of the skin. According to the Cochrane library, heat may be an effective self-care treatment for conditions like rheumatoid arthritis.

Clinical Advantage of Thermotherapy.

A heating pad is one of the best sources for sore necks and backs. Toe warmers, hands and body warmers. It is also portable as relief for Raynauds, lupus, arthritis, myalgia etc. Moist heat can be used on abscesses to help drain the abscesses faster.

Heat therapy also helps to curb the challenge of adverse drug reaction; which is an injury caused by taking a medication. To reduce the percentage of Drug Overdose, misuse, abuse and side effects. ADRs may occur following a single dose or prolonged administration of a drug or result from the combination of two or more drugs (Nebeker *et al*, 2004). A study from 2005 showed heat therapy to be effective in treating cutaneous leishmaniasis tropica (a tropical parasitic skin infection) in Kabul, Afghanistan (Reithinger, 2005). Heat therapy is also sometimes used in cancer treatment to augment the effect of chemotherapy or radiotherapy, but it is not enough to kill cancer cells on its own (Cancer, 2016).

Aromatherapy

Aromatherapy is the treatment or prevention of disease by use of essential oils. Other stated uses include pain and anxiety reduction, enhancement of energy and short-term memory, relaxation, hair loss prevention, and reduction of eczema-induced itching (Kingston, 2010).

Plant Overview

Moringa oleifera is a member of the Moringaceae family of perennial angiosperm plants, which includes 12 other species (Olson, 2010). Native of the sub-Himalayan northern parts of India, it is cultivated throughout tropical and sub-tropical areas of the world, where it is known by various vernacular names, with drumstick tree, horseradish tree and malunggay, being the most commonly found in the literature. *Moringa oleifera* is an edible plant. A wide variety of nutritional and medicinal virtues have been attributed to its roots, bark, leaves, flowers, fruits, and seeds (Anwar *et al*., 2007).

Phytochemical Constituent of *Moringa oleifera* Seeds

The phytochemicals present in the seeds includes saponins, alkaloids, terpenoid, steroids, glycoside, flavonoids (flavone aglycones), and tannins (Fahey, 2005). Phytochemical analyses have also shown that its leaves are particularly rich in potassium, calcium, phosphorous, iron, vitamins A and D, essential amino acids, as well as such known antioxidants such as β-carotene, vitamin C, and flavonoids (Bennett *et al*.,2003).

The seed which have been tagged the most important part of the plant are brownish with semi-permeable seed hull (Makkar and Becker, 1997). It has been used for centuries in traditional medicine to treat a variety of ailments such as arthritis rheumatoid disorders and so on and also to improve overall health in patients due to the presence of chemical substances often referred to as secondary metabolites.

Moringa Seed Oil

Moringa oil is extracted from the seeds of *Moringa oleifera,* by cold press or solvent extraction method. It is pale yellow in color, smells like peanut oil, and is high in behenic acid. Moringa oil has been mentioned as very useful oil in the medicinal books of Greece and Rome. It is great for topical use on the skin and the hair.

Anti-inflammatory Potential

Inflammation is the body's natural response to infection or injury. It is essential as a protective mechanism, but may become a major health issue when it goes on for a long time. Moringa leaves, pods and seeds have been shown to have anti-inflammatory properties as well, which may also be due to isothiocyanates in form of glycosides in the Moringa seeds (Mahajan *et al*., 2007), as a result, the oil reduces inflammation both topically and internally. Moringa oil is a nice rheumatic oil. It is applied to painful, arthritic joints. It is effective at reducing swelling and inflammation, which provides relief from the pain in the joints. It can be use directly, or used as an oil pack, just like castor oil packs.

Clinical Advantage of Aromatherapy

Aromatherapy uses plant materials and aromatic plant oils occurring naturally including essential oils, and other aroma compounds for improving psychological or physical ell-being. It can be offered as a complementary therapy or as a form of alternative medicine. Complementary therapy can be offered alongside standard treatment (Kuriyama*et al*, 2005) with alternative medicine offered instead of conventional, evidence-based treatments. This can be issued through topical application, massage, inhalation or water immersion to stimulate desired response.

Experimental Methods

Sampling of Moringa Seed

The *Moringa oleifera* seed samples were collected from Saminaka, Lere Local Government Area of Kaduna State, Nigeria in 2017 and identified by the Federal College of Forestry, Jos. Plateau State.

The seeds of *Moringa oleifera* were dried for seven (7) days under shade at room temperature to avoid loss of active compounds. The dried seeds were then ground to powder using a mortar and pestle after which it was stored in an air tight vessel for further use.

Aqueous extract: The extraction process used was hot water method following the procedure of Handa (2008). 50g of the powdered sample were soaked in 500cm^3 of distilled water and boiled for about ten minutes. After boiling, the sample was double-filtered using cheese cloth and collected in a conical flask and allowed to cool. The filtrate was then dried in hot air oven at temperature of 70^{o}C.

Ethanol extract: 50g of the powdered sample were soaked in 500cm^3 of absolute ethanol and allowed to stand for 24hrs. The mixture was then stirred up occasionally. After 24hrs the sample was double-filtered using a cheese cloth and collected in a conical flask. The filtrate was dried in hot air oven at temperature of 45^{o}C (Handa, 2008).

I. Extraction of Moringa Seed Oil.

The dried *Moringa oleifera* seeds were subjected to further pounding so as to increase the surface area. About 200g of the Moringa seed powder was set aside for phytochemical analysis, while the remaining was cold-pressed to extract the oil using a wooden mortar. After the cold-press oil extraction process, the cake was discarded while the oil was separated and stored in a bottle.

II. Physico-Chemical Analysis on Moringa Seed Oil.

The physico-chemical analysis of the Moringa seed oil was carried out based on the method specified by Habib (1986) and A.O.A.C (2012).

III. Phytochemical Analysis on Moringa Seed Extract.

The phytochemical analysis of the Moringa seed extract (Aqueous and ethanolic) was carried out to determine the presence of Alkaloids, steroids and triterpenoids, tannins, anthracenosides, flavones aglycones, saponins and coumarins using the standard procedures as described by Chitravadivu *et al* (2009).

IV. Chemical Packs Manufacture

Hot Pad Manufacture from Each Solute

- Anhydrous Sodium Acetate.

A supersaturated solution of sodium acetate was made by adding 50.0 g of anhydrous sodium acetate to 5 cm^3 of water in a small flask. It was then heated past the melting point melt (i.e. 58 °C to 58.4 °C), causing them to dissolve in their water of crystallization and subsequently allowed to cool at room temperature without forming crystals. The supersaturated solution was then poured into HDPE pack and a metallic strip called an activator was placed within the pack and flexed. This causes the solution to crystallize back into solid sodium acetate. The bond-forming process of crystallization is exothermic. The duration was monitored using a stop clock and a thermometer was then used to measure the highest temperature.

- Calcium chloride

  $50cm^3$ of distilled water was poured into a HDPE pack and sealed. Then 20g of Calcium chloride was poured into an empty HDPE pack along with the already sealed water bag and sealed altogether. The sealed bag containing the Calcium chloride and the water was then placed into an empty HDPE pack and sealed. The pack was then activated by popping the inner bag and shaken together. The duration was monitored using a stop clock and a thermometer was then used to measure the highest temperature.

Cold Pad Manufacture from Each Solute

- Ammonium Chloride

  $50cm^3$ of distilled water was poured into a HDPE pack and sealed. Then 20g of ammonium chloride was poured into an empty HDPE pack along with the already sealed water bag and sealed altogether. The sealed bag containing the ammonium chloride and the water was then placed into an empty HDPE pack and sealed. The pack was then activated by popping and shaken together. The duration was monitored using a stop clock and a thermometer was then used to measure the lowest temperature.

- Potassium Chloride

  Then $50cm^3$ of distilled water was poured into a HDPE pack and sealed. Then 20g of potassium chloride was poured into an empty HDPE pack along with the already sealed water bag and sealed altogether. The sealed bag containing the potassium chloride and the water was the placed in an empty HDPE pack and sealed. The duration was monitored using a stop clock and a thermometer was then used to measure the lowest temperature.

V. Determining the Efficacy of Thermo-Aromatherapy

- Study design: the study was an open, randomized trial, in which the efficacy and safety of thermo-aromatherapy was carried out. In most cold packs were applied before hot packs to avoid further inflammation.

- Population and study sites: the study was conducted between February to April, 2017 at Bassa L.G.A of Plateau State. It included adult male subjects who played football regularly.

- Inclusion criteria: Subjects who were included suffered from muscle strain and tear

  Data collection: A voluntary participation form was given to indulge their consent of subject

Results

Table 1: Result Obtained from Physico-Chemical Analysis

S/n	Parameters	Value
1	**Acid value(mg of KOH/1g of oil)**	1.23±0.02
2	**Iodine value(I_2/100g of oil)**	67.87±0.54
3	**Peroxide value($mEqO_2/g$)**	10.80±0.12
4	**Refractive index (25^oC)**	1.4668±0.03

Table 2: Result Obtained from Phytochemical Analysis

S/n	Phytochemical	Aqueous Extract	Ethanolic extract
1	Steroids and terpenoids	++	++
2	Alkaloids	++	-
3	Flavones aglycones	++	+++
4	Anthraquinones	+++	++
5	Tannins	++	+
6	Saponins	++	+
7	Coumarins	-	-

Key: -: not detected; +: present in low concentration; ++: present in moderate concentration; +++: present in high concentrations.

Table 3: Result Obtained from Hot Pad Manufacture Using anhydrous CH_3COONa and $CaCl_2.6H_2O$

Solute													N (mol^{-1})	Q(KJ)	ΔH (KJ/mol)
CH_3COONa	**Temp(° C)**	29	42	44	46	58	58	58	58	54	52	52	0.6095	332.05	544.76
	Time (Min)	0	2	4	6	8	10	12	14	16	18	20			
$CaCl_2.6H_2O$	**Temp(° C)**	29	40	44	50	50	50	46	40	34	33	31	0.1802	1768.12	9811.97
	Time (Min)	0	2	4	6	8	10	12	14	16	18	20			

Table 4: Result Obtained from Cold Pad Manufacture Using NH_4Cl and KCl.

Solute													N (mol^{-1})	Q(KJ)	ΔH (KJ/mol)
NH_4Cl	**Temp(° C)**	29	22	22	23	24	25	25	25	25	26	28	0.3740	470.96	1259.25
	Time (Min)	0	2	4	6	8	10	12	14	16	18	20			
KCl	**Temp(° C)**	29	24	22	22	20	20	23	24	24	24	25	0.2683	3919.8	14609.77
	Time (Min)	0	2	4	6	8	10	12	14	16	18	20			

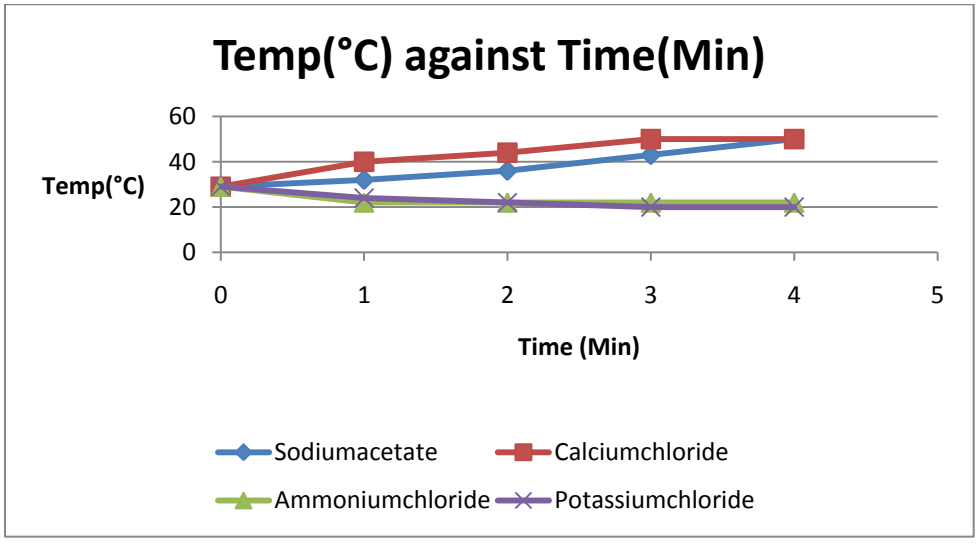
Table 5: Result Obtained From Combining Both Therapeutic Methods

S/n	Subject	Response				Comment
		Day 1	**Day 2**	**Day 3**	**Day 4**	
1	**A**	**-**	**+**	**++**	**++**	Recovered
2	**B**	**-**	**++**	**++**	**+++**	Recovered
3	**C**	**+**	**+**	**++**	**+++**	Recovered
4	**D**	**+**	**+**	**+**	**++**	Recovered
5	**E**	**+**	**++**	**++**	**++**	Recovered
6	**F**	**+**	**++**	**+++**	**+++**	Recovered

Response Key:

-: no response; +: partial response; ++: moderate response; +++: high response to therapy.

Graph 1: A Plot of Temperature Change against Time for Anhydrous CH_3COONa, $CaCl_2$. $6H_2O$, NH_4Cl and KCl



Discussion

I. Moringa oleifera Seed Oil

The oil extracted was a liquid at room temperature, pale yellow in colour and smelled like peanut oil.

The phytochemical analysis was carried out on the seed extract to ascertain the presence of the phytochemicals already mentioned in literature. Thereafter, physicochemical analysis was carried out on the oil sample. The acid value, peroxide value, Iodine value and refractive index of the oil were found to be 1.23±0.02, 10.80±0.12, 67.87±0.54 and 1.4668±0.03 respectively which shows the degree of acidity, rancidity, unsaturation and purity of the oil (Fahey, 2005).

II. Chemical Packs

On activating the anhydrous sodium acetate pack, Heat was evolved and the temperature of the pack rose to about 58° C. Thus, the latent heat of fusion was recorded as 544.76KJ/mol. When the Calcium Chloride pack was popped, heat was evolved and the temperature of the pack rose to about 50° C. Thus, the heat of dissolution is being recorded as 9811.97KJ/mol. When the Ammonium chloride and potassium chloride pack were popped, Heat was absorbed and the temperature of the pack dropped from 29° C (room temperature) to 22° C and to 20° C with heat of dissolution recorded as 1259.25KJ and 14609.77KJ as shown on the graph respectively.

III. Thermo-Aromatherapy

Thermo-therapeutic treatments depend on the type of disease. There are three phases of the healing process:

The inflammatory phase: which may last for about two days; during this phase, cold packs are usually applied and the injured area is protected from further injury while the body contains the damaged tissue.

The proliferation phase: during this phase new tissue and scar tissue are formed. Heat can now be applied to the area to facilitate the healing.

The remodeling phase: during this phase, restoration of structure and function of the injured tissues using heat therapy (Kathleen *et al*., 1999). Usually, subjects under thermotherapy require several weeks to recover. However, at the end of the trials, the subjects showed a positive response to thermo-aromatherapy, with recovery duration of about four (4) days.

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Conclusion

From the research work carried out, thermo-aromatherapy offers an effective and safer, alternative for pain relief, they are convenient, easy to use and provides effective therapy that complies with medically accepted protocols which are; free of addiction potential, does not cause skin problems, used safely by women who are pregnant, does not cause skin problems such as contact dermatitis, used safely by women with menstrual pain as researched by French *et al*., (2006).

Thermo-aromatherapy can also serve as an alternative method of treating pain in diabetics and for those who are allergic to oral medication (pharmacophobia), or who for one reason or the other, do not subscribe to oral medication.

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