

Complaint to TGA: Nature's Sunshine Andrographis

Complaint Summary

Nature's Sunshine Products of Australia Pty Ltd makes the following claims on their web site for their Andrographis product (ARTG 302367):¹

1. 'Contains a specific extract which has been scientifically shown to assist in reducing the severity of Mild Upper respiratory tract infection symptoms within 3 days. Saxena RC Et al. (2010) Phytomedicine. Mar;17(3-4):178-85'.
2. 'Reduces symptoms of sinusitis'.

The public summary permitted indications of this product (appended) also states, 'Decrease/reduce/relieve symptoms of sinusitis' and 'Helps reduce occurrence of symptoms of upper respiratory tract infections' and 'Helps decrease/reduce/relieve mild swelling of the glands associated with common cold'.

I allege these claims breach s. 9(a)&(b) and s.15(3)(a) of the Therapeutic Goods Advertising Code (No.2) 2018.

Complaint details

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See also:

- <https://www.vitalityandwellness.com.au/products/natures-sunshine-andrographis>
- <https://www.discountvitaminsexpress.com.au/product/natures-sunshine-andrographis-60-vege-capsules>
- <https://www.obornehealth.com.au/Natures-Sunshine-Andrographis-60c>
- <https://www.preciousorganics.com.au/products/naturessunshineandrographis60s>
- <https://www.superpharmacy.com.au/products/natures-sunshine-andrographis-60-capsules>
- Etc.

First, the paper by Saxena et al., (2010) appended, noted that in both the placebo and KalmCold™ groups the mean scores of all symptoms showed a similar decreasing trend from day 1 to day 3. A statistically significant difference between the placebo and KalmCold™ treatment arms did not occur until day 5. **Thus, the claim that the Andrographis extract reduces URTI symptoms within 3 days is not in accord with the reference cited.**

¹ <https://www.naturessunshine.com.au/products/andrographis>

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Second, the claim that the Andrographis extract reduces symptoms of sinusitis was also not supported in the reference cited; this study specifically excluded subjects suffering from sinusitis.

Third, there was no mention of 'reducing the occurrence of symptoms' or 'relieving mild swelling of the glands' in the paper.

These claims breach s. 9(a)&(b) of the Therapeutic Goods Advertising Code (No.2) 2018.

Fourth, the research conducted by Saxena et al., was co-authored Natural Remedies Pvt. Ltd., who also provided technical assistance. This company manufacture and distribute (sell) the specific extract (Ap BIO®-Kalm cold®) which Carl Gibson said (appended)was used in the Nature's Sunshine product.² The Saxena paper has no conflict of interest statement, or any information about funding, but it is reasonable to assume that Natural Remedies Pvt. Ltd was a financial sponsor of this research.

I suggest that this information should have been included in the advertisement (and ingredient information of the Public Summary); its absence in the advertisement is a breach of s.15(3)(a) of the Therapeutic Goods Advertising Code (No.2) 2018.

Fifth, the study was randomized, double blind and placebo controlled, with symptoms assessed by the study subjects using a Visual Analogue Scale(VAS) from 0-100 where the value '0' indicated no symptoms and '100' meant the highest severity of symptoms. The paper made no mention of whether blinding was checked, for example by asking the study subjects if they thought they were receiving the placebo or active product. All plant parts of Andrographis paniculata have an extremely bitter taste (the king of bitters).³ While encapsulation may have camouflaged this property, blinding should have been checked.

This limitation of the study, together with concerns about the involvement of Natural Remedies Pvt. Ltd., casts doubt on the validity of the results, in particular the claim, 'scientifically shown to assist in reducing the severity of Mild Upper respiratory tract infection symptoms'. This study needs independent replication.

Finally, a 2017 systematic review and meta-analysis of Andrographis paniculata (Chuān Xīn Liá'n) for the symptomatic relief of acute URTI in adults and children has been reported.⁴ Thirty-three RCTs (7175 patients) were tabulated, including the Saxena trial. Most trials evaluated Andrographis paniculata as commercial monotherapy, or herbal mixtures, but they seldom reported manufacturing or quality control details.

The authors concluded that Andrographis paniculata appeared beneficial and safe for relieving ARTI symptoms and shortening time to symptom resolution. **However, they noted these findings should be interpreted cautiously owing to poor study quality and heterogeneity.** Well-designed trials evaluating the effectiveness and potential to reduce antibiotic use of Andrographis paniculata are warranted. **It would also be nice to be reassured that the extract (Ap BIO®-Kalm cold®), said to be used in the Nature's Sunshine product, was shown to have batch to batch consistency with that used in the Saxena trial.**

² <https://www.giellepi.com/partner/natural-remedies/>

³ <https://ndnr.com/botanical-medicine/looking-more-closely-at-andrographis-paniculata/>

⁴ <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5544222/>

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In conclusion, I remain to be convinced that Nature's Sunshine Andrographis product, 'has been scientifically shown to assist in reducing the severity of Mild Upper respiratory tract infection symptoms'.

Dr Ken Harvey MBBS, FRCPA, AM
Associate Professor
School of Public Health and Preventive Medicine

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Email: kenneth.harvey@monash.edu
WWW: www.medreach.com.au
6 August 2020

Screen shots taken 06/08/2020

Home / Andrographis

NATURE'S SUNSHINE Shop Health Topics Store Locator Ask a Naturopath About

Sold Out

Andrographis

A\$37.95 +

Nature's Sunshine Andrographis contains a specific extract which has been scientifically shown to assist in reducing the severity of Mild Upper respiratory tract infection symptoms within 3 days*.

- o Mild Upper respiratory tract infection Symptoms
- o Common cold
- o Maintains and supports healthy immune system function

This medicine may not be right for you. Read the warnings before purchase.

ANDROGRAPHIS
Immune Support, Common Cold
Mild Upper Respiratory Tract Infection Symptoms
60 VEGE CAPSULES

<https://www.naturessunshine.com.au/products/andrographis>

Complaint to TGA: Nature's Sunshine Andrographis

CAUTIONS

Not recommended while pregnant or breast feeding.
Take two hours away from all prescription medications

WARNINGS

Always read the label. Follow directions for use. If symptoms persist, talk to your health professional. Adults only. Andrographis may cause allergic reactions in some people. If you have a severe reaction (such as anaphylaxis) stop use and seek immediate medical attention.

*Saxena RC Et al. (2010) Phytomedicine. Mar;17(3-4):178-85.

<https://www.naturessunshine.com.au/products/andrographis>

Nature's Immune Support

- Relieves symptoms and duration of the common cold
- Maintains healthy immune function
- Relieves symptoms of mild upper respiratory tract infections
- Reduces symptoms of sinusitis



Fresh andrographis paniculata leaves.

EACH CAPSULE CONTAINS

Herbal extract equivalent to:
Andrographis paniculata (Andrographis) dry leaf 1.4g (1400mg)
Equivalent to Andrographolide 35mg

DOSAGE

Adults: Take one capsule twice daily, or as professionally prescribed.

PACK SIZE

60 vege capsules

NO ADDED

Vegan friendly. No added animal products, gluten, lactose, soy, starch, sugars or salt, wheat products, artificial colourings, flavourings or preservatives.

<https://www.naturessunshine.com.au/products/andrographis>

Complaint to TGA: Nature's Sunshine Andrographis

Correspondence

----- Forwarded message -----

From: **Kenneth Harvey** <kenneth.harvey@monash.edu>
Date: Tue, 4 Aug 2020 at 11:42
Subject: Nature's Sunshine Andrographis
To: <contact@nspaust.com.au>

Dear Nature's Sunshine people,

Your web site: <https://www.naturessunshine.com.au/products/andrographis> states:

'Nature's Sunshine Andrographis contains a specific extract which has been scientifically shown to assist in reducing the severity of Mild Upper respiratory tract infection symptoms within 3 days. Saxena RC Et al. (2010) Phytomedicine. Mar;17(3-4):178-85'.

Could you please confirm that the extract used in your product is identical to that used in the paper cited. That is, does it contain KalmCold TM with the following chromatogram?

Sincerely,
Ken

----- Forwarded message -----

From: **Adele Ghraiche** <adele@nspaust.com.au>
Date: Tue, 4 Aug 2020 at 14:03
Subject: RE: Nature's Sunshine Andrographis
To: kenneth.harvey@monash.edu <kenneth.harvey@monash.edu>

Hi Ken,

Thank you for taking the time to contact us.

We have referred your enquiry to our industry association, and they will contact you.

Kind regards,

Adele Ghraiche ND, BAppSc Nat, PGradDip Nat
Technical & Regulatory Affairs Manager
P: 02 9894 0111
F: 02 9894 2422
E: adele@nspaust.com.au
W: naturessunshine.com.au

On Wed, 5 Aug 2020 at 12:11, Carl Gibson <carl.gibson@cmaustralia.org.au> wrote:

G'day Ken,

I am pleased to confirm that Nature's Sunshine are members of Complementary Medicines Australia.

As a smaller sponsor their preference is that I respond directly to the question raised.

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After reviewing the issue I can confirm that Nature's Sunshine use a clinically proven standardized phytochemical composition derived from Andrographis, a branded ingredient of Natural Remedies Pvt Ltd and is registered in the US and Australia under the trade mark AP-Bio.

For clarity, AP-Bio is a registered trade mark of KalmCold, and are one and the same.
(Happy to provide the certification If that helps)

Hope that helps clarify.

Carl



Australian Government
Department of Health
Therapeutic Goods Administration

Public Summary

Summary for ARTG Entry:	302367	Nature's Sunshine Andrographis
ARTG entry for	Medicine Listed	
Sponsor	Nature's Sunshine Products of Australia Pty Ltd	
Postal Address	PO Box 6884, BAULKHAM HILLS BUSINESS CENTRE, NSW, 2153 Australia	
ARTG Start Date	27/04/2018	
Product Category	Medicine	
Status	Active	
Approval Area	Listed Medicines	

Conditions

Colouring agents used in listed medicine for ingestion, other than those listed for export only under section 25 of the Act, shall be only those included in the list of 'Colourings permitted in medicines for oral use'.

The sponsor shall keep records relating to this listed medicine as are necessary to: (a) Expedite recall if necessary of any batch of the listed medicine, (b) Identify the manufacturer(s) of each batch of the listed medicine. Where any part of or step in manufacture in Australia of the listed medicine is sub-contracted to a third party who is not the sponsor, copies of relevant Good Manufacturing Practice agreements relation to such manufacture shall be kept.

The sponsor shall retain records of the distribution of the listed medicine for a period of five years and shall provide the records or copies of the records to the Complementary Medicines Branch, Therapeutic Goods Administration, upon request.

The sponsor of the listed medicine must not, by any means, intentionally or recklessly advertise the medicine for an indication other than those accepted in relation to the inclusion of the medicine in the Register.

The sponsor shall not supply the listed medicine after the expiry date of the goods.

Where a listed medicine is distributed overseas as well as in Australia, product recall or any other regulatory action taken in relation to the medicine outside Australia which has or may have relevance to the quality, safety or efficacy of the goods distributed in Australia, must be notified to the National Manager Therapeutic Goods Administration, immediately the action or information is known to the sponsor.

Products

1 . Nature's Sunshine Andrographis

Product Type	Single Medicine Product	Effective Date	10/06/2020
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Permitted Indications

Traditionally used in Ayurvedic medicine to bitter tonic/stimulate gastric secretions

Maintain/support healthy immune system function

Decrease/reduce/relieve common cold duration

Decrease/reduce/relieve mild upper respiratory tract congestion

Decrease/reduce/relieve the severity of symptoms of mild upper respiratory tract infections

Linked indication - Decrease/reduce headache duration

Linked indication - Relieve feelings of general malaise/general debility

Linked indication - Relieve runny/dripping nose

Linked indication - Decrease/reduce/relieve cough

Linked indication - Decrease/reduce/relieve mild throat inflammation

Linked indication - Unblock/clear nasal passages

Linked indication - Decrease/reduce/relieve disturbed/restless sleep

Decrease/reduce/relieve symptoms of sinusitis

Linked indication - Decrease/reduce/relieve sneezing

Decrease/reduce/relieve the severity of common cold symptoms

Relieve symptoms of mild upper respiratory tract infections

Helps reduce occurrence of symptoms of upper respiratory tract infections

Decrease/reduce/relieve symptoms of common cold

Linked indication - Helps decrease/reduce/relieve mild swelling of the glands associated with common cold

Indication Requirements

Label statement: Adults only, OR Not to be used in children under 2 years of age without medical advice (or words to that effect).

Label statement: If symptoms persist, talk to your health professional.

Product presentation must not imply or refer to serious immunological diseases.

Product presentation must not imply or refer to serious forms of respiratory disorders/diseases, such as: asthma, pneumonia, COAD, COPD, influenza.

Respiratory tract infections must be qualified by 'mild'.



Australian Government
Department of Health
Therapeutic Goods Administration

Product presentation must not imply or refer to mental illnesses, disorders or conditions.

Product presentation must not imply or refer to chronic fatigue syndrome.

Product presentation must only refer to mild gland swelling.

Standard Indications

No Standard Indications included on Record

Specific Indications

No Specific Indications included on Record

Warnings

If symptoms persist consult your healthcare practitioner (or words to that effect).

Andrographis may cause allergic reactions in some people. If you have a severe reaction (such as anaphylaxis), stop use and seek immediate medical attention (or words to that effect).

Adults only (or words to that effect).

Additional Product information

Pack Size/Poison information

Pack Size

Poison Schedule

Components

1 . Formulation 1

Dosage Form Capsule, hard

Route of Administration Oral

Visual Identification

Active Ingredients

Andrographis paniculata 100 mg

Other Ingredients (Excipients)

calcium carbonate

calcium hydrogen phosphate dihydrate

colloidal anhydrous silica

hypromellose

magnesium stearate

purified water

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Public Summary



A randomized double blind placebo controlled clinical evaluation of extract of *Andrographis paniculata* (KalmCold™) in patients with uncomplicated upper respiratory tract infection

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^e Research Division, ASA Foundation, Gurgaon, India

^f Research and Development Centre, Natural Remedies Pvt. Ltd., Bangalore, India

ARTICLE INFO

Keywords:

Andrographis paniculata

KalmCold™

Upper respiratory tract infection

Common cold

Efficacy

Double blind placebo controlled trial

ABSTRACT

A randomized, double blind placebo controlled clinical study was conducted to evaluate the efficacy of KalmCold™, an extract of *Andrographis paniculata*, in patients with uncomplicated upper respiratory tract infection (URTI). The assessment involved quantification of symptom scores by Visual Analogue Scale. Nine self evaluated symptoms of cough, expectoration, nasal discharge, headache, fever, sore throat, earache, malaise/fatigue and sleep disturbance were scored. A total of 223 patients of both sexes were randomized in two groups which received either KalmCold™ (200 mg/day) or placebo in a double blind manner. In both the treatments, mean scores of all symptoms showed a decreasing trend from day 1 to day 3 but from day 3 to day 5 most of the symptoms in placebo treated group either remained unchanged (cough, headache and earache) or got aggravated (sore throat and sleep disturbance) whereas in KalmCold™ treated group all symptoms showed a decreasing trend. Within groups, mean scores of symptoms in both the groups decreased significantly ($p \leq 0.05$) from day 1 to day 3 and day 5 while from day 3 to day 5 all symptoms except expectoration in placebo group did not improve significantly whereas in KalmCold™ treated group all symptoms improved significantly ($p \leq 0.05$) except earache. Comparing mean between both groups, all symptoms at day 1 and day 3 were found to be the same while at day 5 all symptoms except earache in KalmCold™ treated group improved significantly ($p \leq 0.05$) than placebo group. Similarly, within groups, overall scores of all symptoms in both the groups decreased significantly ($p \leq 0.05$) from day 1 to day 3 and day 5 while from day 3 to day 5 placebo group did not improve significantly whereas KalmCold™ treated group showed significant improvement ($p \leq 0.05$). On between groups analysis, KalmCold™ group showed significant reduction ($p \leq 0.05$) in overall symptom scores as compared to placebo group. In both placebo and KalmCold™ treated groups, there were only a few minor adverse effects with no significant difference in occurrence ($Z = 0.63$; $p > 0.05$). The comparison of overall efficacy of KalmCold™ over placebo was found to be significant ($p \leq 0.05$) and it was 2.1 times (52.7%) higher than placebo. The findings of this study revealed that KalmCold™ was effective in reducing symptoms of upper respiratory tract infection.

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Introduction

Common cold (synonyms: acute coryza, acute viral nasopharyngitis) is a highly contagious, viral disease of the upper respiratory tract. More than 200 different viruses are reported as etiological agents for common cold and flu (NIAID, 2007) and among them rhino virus is reported to be the most common causative agent in humans (Hershenson and Johnston, 2006).

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E-mail addresses: joshua@naturalremedy.com, jjoshuaallan@gmail.com, pharmacology@naturalremedy.com (A.J. Joshua).

Upper respiratory tract infection (URTI) is recognized as leading cause for absence from school and work (Melchior et al., 1997). As per the estimates of the Centers for Disease Control and Prevention, 22 million school days are lost annually in the United States due to the common cold (CDC, 2004). The general symptoms of URTI include cough, expectoration, nasal discharge, headache, fever, sore throat, earache, malaise/fatigue and sleep disturbance (Caceres et al., 1999; Eccles, 2005). No specific antiviral therapy or vaccination against the common cold is available at present. The existing symptomatic treatment with antibiotics, nasal decongestants, cough suppressants, anti histamines and analgesics is found to be of limited value as the agents mainly focus on relieving the symptoms rather than treat the condition (Caceres et al., 1999).

A vast number of medicinal plants of traditional use are reported to exhibit great potential of therapeutic applications and botanical products are being considered as important part of the health food market (Schiliter et al., 2003). *Andrographis paniculata* Nees (*A. paniculata*) commonly known as Chiretta, King of Bitters or Kalmegh, has been widely used in traditional system of medicine in India. *A. paniculata* belonging to family Acanthaceae, is an annual herb native to peninsular India and Sri Lanka and is also distributed in different regions of South-east Asia, China, America, West Indies and Christmas Island (Lattoo et al., 2006). *A. paniculata* has been shown to possess wide spectrum of pharmacological properties (Mishra et al., 2007; Khare, 2007).

Herbal preparations that contain extract of *A. paniculata* as their principal ingredient are reported to improve the recovery from common cold and reduce its occurrence. The beneficial effects of *A. paniculata* in the management of common cold have been clearly validated in several clinical trials of recent past (Thamlikitkul et al., 1991; Melchior et al., 1997; Caceres et al., 1997; Melchior et al., 2000) and meta analyses of the clinical trials also pointed out to its favourable effectiveness (Coon and Ernst, 2004; Poolsup et al., 2004). Recent research has indicated that cold remedies which exhibit anti-inflammatory and antiviral milieu in the respiratory tract are imperative in the treatment of common cold (Mossad, 1998). The primary mechanisms of action of *A. paniculata* are attributed to the anti-inflammatory (Shen et al., 2002) and immunostimulant (Puri et al., 1993; Kumar et al., 2004) properties of its main active ingredient – Andrographolide. *A. paniculata* is listed in Indian Herbal Pharmacopoeia, Herbs of Commerce of American Herbal Products Association, and in Indian Pharmacopoeia (IDMA, 2002; AHPA, 2004; IPC, 2007). It has been used for long without any known toxicity and has a strong traditional usage from safety point of view (Puri, 2003). The available human studies on *A. paniculata* did not indicate any serious adverse effects (Coon and Ernst, 2004).

With the above perspectives, the study was conducted to evaluate the efficacy of extract of *A. paniculata* (KalmCold™) in patients with uncomplicated upper respiratory tract infection.

Materials and methods

Study design

The study was conducted at four different centers in India between May 2007 and November 2007. Due to the self-limiting nature of the illness, it was decided to introduce a placebo group and carry out the study in a double blind manner.

Investigational substance

The investigational substance used was capsule of KalmCold™, an extract from the leaves of *A. paniculata* Nees, developed by M/s Natural Remedies Pvt. Ltd., Bangalore, India. Each capsule of KalmCold™ (Sample code: 26 (APE-30); Batch No.: AP07/Lot 03) contained *A. paniculata* extract 100 mg and 200 mg of micro crystalline cellulose. The extract contained andrographolide (31.30% w/w), isoandrographolide (0.40% w/w), neoandrographolide (3.20% w/w), andrograpanin (0.60% w/w), skullcapflavone I (0.05% w/w), 7-O-methylwogonin (0.05% w/w) and total andrographolides (38.40% w/w). The content of 14-deoxy 11, 12, didehydroandrographolide was 2.80% w/w. The composition adheres to the international quality requirements which include analysis of solvent residue, heavy metals residue, mycotoxin residue, pesticide residue evaluation and microbial contamination. The placebo capsules (Sample code: 27 (APE-30)) contained 300 mg of micro crystalline cellulose. The test medication and the placebo were filled in 0 size blue hard gelatin capsules that could not be distinguished from each other. Both investigational substance and the placebo capsules were packed in blisters of sample size 10 capsules/blister pack and labeled with code numbers.

Preparation of KalmCold™

Coarse ground leaves of *A. paniculata* (300 kg) were charged into a stainless steel jacketed extractor fitted with a reflux condenser. Methanol (1200 L) was added to the extractor and the contents were refluxed for 3 h by providing steam in the jacket. The liquid extract was drained from the extractor into a separate vessel and fresh methanol (1000 L) was added to the extractor containing the marc. The extraction procedure as above was repeated two more times and the liquid extracts from each extraction step was separately subjected to distillation under vacuum (at $\leq 55^\circ\text{C}$) until a thick paste with a total solid content of 40–50% (w/w) was obtained. Thick paste obtained from the three extraction steps were mixed and dried under vacuum ($\leq 65^\circ\text{C}$) to get lumps of the extract. The extract lumps were then milled and sieved (# 40) to get a uniform powdered extract of *A. paniculata* (18 kg). To the marc contained in the extractor, water (1200 L) was added and the contents were refluxed for 3 h by providing steam in the jacket. The liquid extract was drained from the extractor into a concentrator and was subjected to distillation under vacuum (at $\leq 75^\circ\text{C}$) until the total solid content in the liquid reached about 15–20% (w/v). The concentrated liquid was then spray dried to get the water extract of *A. paniculata* (10 kg). The alcohol and water extracts were then analyzed for the content of active constituents and blended to get KalmCold™ with the required levels of active constituents.

Analysis

KalmCold™ was subjected to physico-chemical and phytochemical analysis. The chemical analysis revealed that the moisture content was 4–5% (USP, 2007a), ash was 10% (USP, 2007b) and total bitters about 60% (IP, 1966) accounting to almost 75% of the composition. KalmCold™ on analysis by HPLC was found to contain the following constituents, viz., andrographolide ($> 30.0\%$ w/w), isoandrographolide ($> 0.3\%$, w/w), neoandrographolide ($> 1.0\%$, w/w), andrograpanin ($> 0.3\%$, w/w), 14-deoxy-11,12-didehydroandrographolide ($\leq 5.0\%$, w/w), skullcapflavone I ($> 0.05\%$, w/w) and 7-O-methylwogonin ($> 0.05\%$, w/w). One of the above constituents viz., 14-deoxy-11,

12-didehydroandrographolide has been reported to cause dose dependent hypotension (Zhang et al., 1998; Yooan et al., 2007). Hence, content of 14-deoxy-11, 12-didehydroandrographolide was constrained to an upper limit of less than 5% w/w.

Isolation of phytochemical reference standards

The extract was subjected to liquid-liquid partitioning between ethyl acetate and water. The ethyl acetate layer was repeatedly chromatographed over silica gel using combinations of hexane: ethyl acetate and chloroform: methanol. Crystallization of different chromatographic fractions yielded andrographolide (**1**), isoandrographolide (**2**), neoandrographolide (**3**), andrograpalin (**4**), 14-deoxy-11, 12 didehydroandrographolide (**5**), skullcapflavone I (**6**) and 7-O-methylwogonin (**7**). Identification of these compounds was confirmed by comparing their ¹H and ¹³C NMR data with literature (Jalal et al., 1979; Fujita et al., 1984; Kuroyanagi et al., 1987; Matsuda et al., 1994; Jayakrishna et al., 2001). Purity of the isolated compounds was determined by thin layer chromatography (TLC) and high performance liquid chromatography (HPLC) for their use as reference standards. The HPLC purity of the isolated constituents was found to be >98.0% by area normalization.

Standard and sample solution preparation

Standards (**1-7**) were prepared by weighing 10 mg of the individual standards and dissolving them separately in 10 ml with methanol (HPLC grade, Qualigens make). The stock solution was suitably diluted to obtain 0.5 mg/ml of **1** and 0.1 mg/ml of **2-7** as a mix and individual solution. Sample solution was prepared by dissolving 200 mg of KalmCold™ in 100 ml of methanol.

Methodology

HPLC method was adopted for analyzing **1-7** compounds. The analytical method was validated for specificity, linearity, precision, accuracy, and range of quantification (ICH, 2005). A known volume (20 µL) of the individual, mixed standard and sample solutions were injected to the HPLC system (Shimadzu, Model LC 2010 A, Japan) consisting of quaternary pump with UV detector, auto injector and column oven with class LC solution software. The stationary phase was an octadecylsilane column (C18, 5 µm, 250 x 4.6 mm, Hibar RT, Lichrosphere 100, Merck). The mobile phase consisted a mix of phosphate buffer (Solvent A) [prepared by dissolving 0.136 g of potassium dihydrogen orthophosphate (KH₂PO₄) in 900 ml of HPLC grade water (obtained from "Arium" Sartorius water purification system) and 0.5 ml of orthophosphoric acid (H₃PO₄) (AR grade, Rankem) then the final volume was made upto 1000 ml] and acetonitrile (Solvent B) (HPLC grade, Qualigens make). Both acetonitrile and the phosphate buffer was filtered separately through 0.45 µm membrane filter and degased by sonicating for 3 min. The solvent A and B were mixed in such a manner that the concentration of solvent B was increased from 5 - 45% as linear gradient in the first 18 min. From 18 to 25 min. the concentration of solvent B was increased from 45 - 80% as a linear gradient. The flow rate of mobile phase was maintained at 1.5 ml per minute throughout the analysis and the detector wave length was kept at 223 nm, and chromatogram was recorded. Quantification of **1** to **7** was achieved by external standard method. The peaks in the mixed standards and in KalmCold™ were identified by injecting the individual standard solutions (Fig. 1).

Participants

The required sample size for difference between two means i.e. for a two sample t-test was estimated according to *Snedecor and Cochran* (1989). The required sample size for each arm of KalmCold™ or placebo was a minimum of 92 subjects or a total 184 for the whole study. As drop outs are common in clinical trial of a self-limiting condition, around 20% more subjects were added in each group. Two hundred and twenty three participants of both sexes (143 males and 80 females) aged between 18 and 60 years were enrolled for the study. The clinical symptoms of upper respiratory tract infection are characteristically heterogeneous in measurement (*Poolsup et al., 2004*) and are categorized as 'early' or 'later' symptoms based on the occurrence/onset, which varies from immediate to several days (*Eccles, 2005*). Such symptoms of common cold varies the sample size between individual symptom scores. Based on this, the sample size of this study was calculated considering any difference in the overall symptoms scores between the two treatments.

Informed consent

The objectives along with detailed procedures of the study were explained to all the participants in their own simple, communicable language. All the participants were requested to understand and sign the informed consent form before randomization procedure. All these patients were provided the clinical symptom assessment/Visual Analogue Scale (VAS; scale from 0-100 wherein the value '0' indicates no symptoms and '100' meaning the highest severity of symptoms) presented in local language or English for ease of understanding of the evaluation procedure. In addition, any queries/doubts, raised by trial subjects prior to signing the consent form were clarified by the investigator. Copy of the informed consent was given to the subjects participating in the study. Consent was taken by the investigators of the clinical trial.

The patients were recruited according to the following inclusion and exclusion criteria:

Inclusion criteria

- Subjects in the age group of 18–60 years, of either sex.
- Subjects who have given written informed consent.
- Subjects predominantly suffering from two or more symptoms of common cold (cough, expectoration, running nose, headache, fever, sore throat, earache, malaise/fatigue and sleep disturbance).
- Subjects agreed to come for follow-up on day 3 and day 5 irrespective of any relief or side effects.

Exclusion criteria

- Patients who were unable to give voluntary consent.
- Patients who were ill more than three days with common cold
- Patients with diabetes or with serious ailments of heart, liver, kidney or brain
- Subjects suffering from immunologically associated diseases such as multiple sclerosis, polyarthritis or rheumatic or other autoimmune diseases.
- Subjects suffering from accompanying illnesses such as bronchitis, pneumonia, pleuritis, septic infections, special bacterial infections as pneumoconiosis, angina tonsilaris, sublingual fever higher than 40.5 °C, sinusitis or any other infections.
- Subjects who had any allergy or were allergic to any medication.

- Patients who were pregnant or lactating.
- Subjects who were unable to complete follow-up on day 3/5.
- Subjects on any medication that would affect evaluation like anti-histamines, steroids, Over-the-counter (OTC)

- or herbal medicines for control of the symptoms of URTI.
- Patients addicted to smoking, tobacco/pan masala chewing or alcohol.

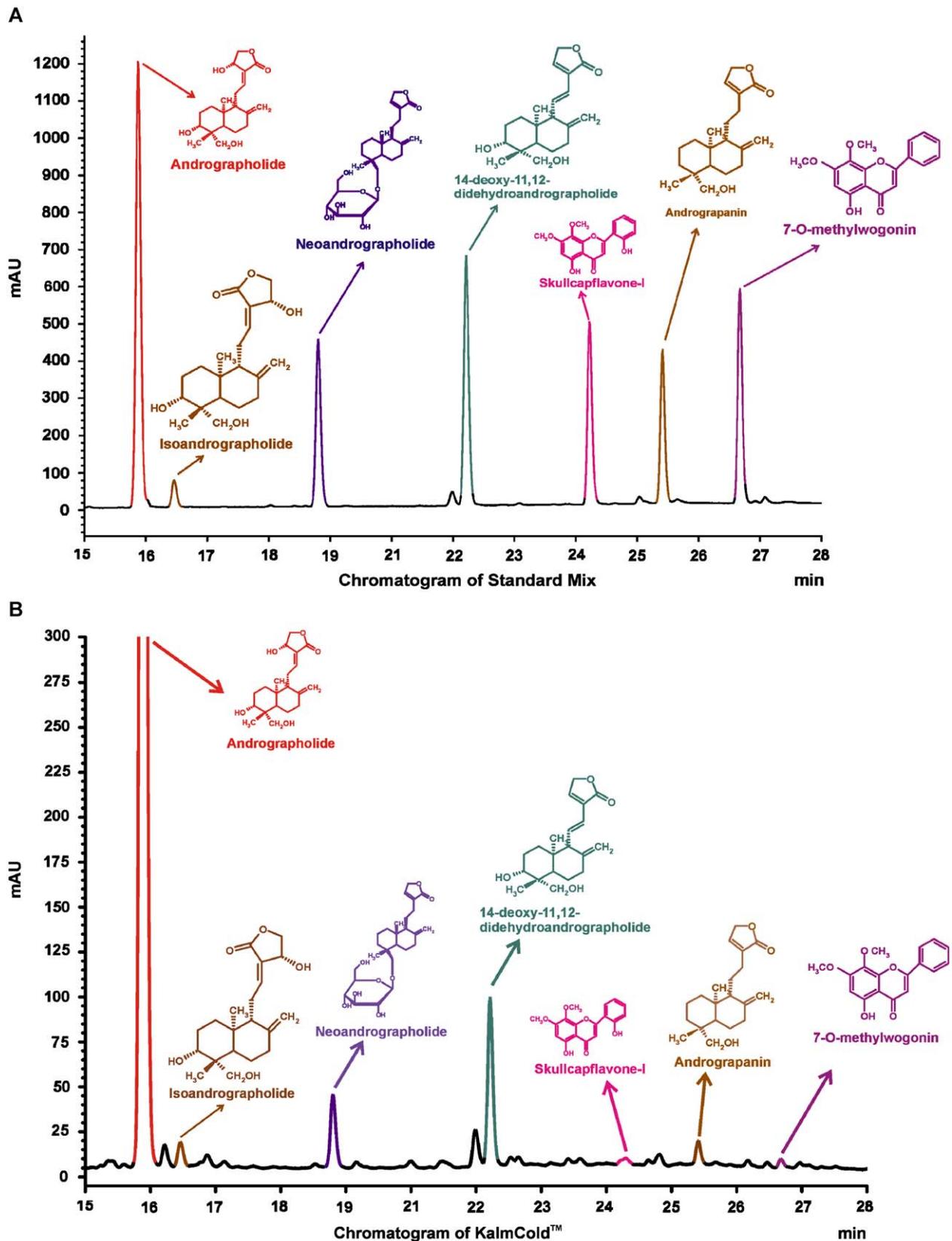


Fig. 1. HPLC chromatogram of standard mix (A) and KalmCold™ (B).

Randomization

The subjects enrolled for clinical trial were allotted to placebo and KalmCold™ groups using a simple randomization procedure. A computer aided random series programme was used to generate the random allocation sequence, which is a list of unique integer random numbers identified as patient code. The unique integer random numbers were then mentioned in respective places (blister pack containing either placebo or KalmCold™) as per the random allocation sequence. The random allocation sequence was generated at Natural Remedies, Bangalore from where the labeled and packed materials for clinical trial were dispatched to study centre. The entire process was carried out in completely concealed manner and all concerned in study centre viz., investigator, participants and staff were unaware of the sequence. The participants fulfilling the inclusion/exclusion criteria of the study and after obtaining the written informed consent were enrolled by the study investigator at the study centre and subsequently the pharmacist dispensed the study medication to the participants taking into consideration the order of enrolment and as per the random allocation sequence. The investigator, participants and pharmacist dispensing the interventions were all blinded to group assignment. The blinding process was maintained till all the data were compiled and confirmed for accuracy and then forwarded to the statistician for analysis.

Dosage regimen and dose rationale

Each patient was handed over one pack containing 10 capsules. Each patient was advised to take one capsule containing 100 mg of actives with a glass of water twice a day after breakfast and dinner (total of 200 mg of actives per day) for a period of five days. The dosage was based on the published clinical trials carried out on *A. paniculata* for the mentioned indication.

Symptomatic assessment of efficacy

Symptoms were assessed on day 1, 3 and 5 of treatment period. Each individual was requested for his/her availability to the investigator for routine follow up and for final clinical examination. The following outcome measures were assessed on each occasion: Cough, expectoration, nasal discharge (running nose), headache, fever, sore throat, earache, malaise/fatigue and sleep disturbance. The patient was asked to grade each individual symptom on VAS. On subsequent follow up, the participants were shown a fresh sheet on which their symptoms were re-graded without referring to the previous rating. The scores of each symptom present on day 1, 3 and 5 of assessment were recorded. The improvement or otherwise was assessed by the changes in the scores over subsequent days.

Safety evaluation

A record of all adverse events reported by the patients was maintained. Patients in the study were instructed and or specifically asked to report to the investigators in the event of any adverse events which the patient or the investigator may attribute to the test substance treatment during start of treatment and follow up on day 3 and day 5.

Restrictions

The study imposed restriction of co-administration of any other drugs viz. antibiotics, cough remedies, anti-histamines or

any other herbal/OTC product for control of the symptoms of URTI. No special diet/change in dietary pattern and activity was advised during the study period. Subjects were requested not to participate in any other clinical study during the trial period.

Statistics

Two hundred and twenty participants were considered for the final statistical analysis. Patients' characteristics of two groups were compared by independent samples t-test. Individual symptom scores recorded at different time intervals for each group (Placebo and KalmCold™) were analyzed using paired samples t-test (within groups). Similarly, independent samples t-test was applied to compare the individual symptom scores between the groups. For each periods and treatments, the overall symptoms scores were calculated and for this, each symptom score in an individual was pooled on a particular day and was considered as total symptom score of that individual. The average of total symptom of all the subjects was then calculated and considered as mean scores of overall symptoms. The overall symptom score were chosen to overcome the clinical heterogeneity in the measurement and scoring of symptoms of upper respiratory tract infection (Poolsup et al., 2004). Similarly, the effect on each symptom and overall symptoms is the mean difference of respective symptoms scores at day 1 and day 5. The effect size is the mean difference of effect between placebo and KalmCold™. To interpret the results, a percent mean change of effect of KalmCold™ over placebo was evaluated as

$$\text{Effect size(\%)} = [(KalmCold^{\text{TM}} - \text{Placebo}) / KalmCold^{\text{TM}}] \times 100$$

In patients, wherein the overall symptoms scores was increased from day 1 to day 5 were considered as 'Aggravated' while in subjects the overall symptoms scores remain same at day 1 and day 5 as 'Unchanged'. The symptoms severity of patients and adverse effects observed in two groups were analysed by proportion Z test using correction for continuity (Zar, 1974). The above statistical applications were performed using SPSS software. A two-tailed ($\alpha=2$) probability value $p \leq 0.05$ was considered to be statistically significant.

Results

Patients' characteristics

Out of 234 subjects assessed for eligibility, a total of 223 patients who fulfilled the selection criteria and willing to give informed consent were enrolled in the study and randomized to either placebo (n=111; 68 males and 43 females) or KalmCold™ (n=112; 75 males and 37 females) groups. The demographic characteristics of all available patients are summarized in Table 1. On comparison, mean characteristics of both the groups at baseline (day 1) were found to be similar.

Drop-outs

Out of 223 enrolled patients at baseline, three patients in placebo group did not turn up for follow up and hence excluded from analysis of symptom scores. Since the number of subjects in both the groups were almost similar it was considered appropriate to exclude them from overall assessment as justified in practice. Thus symptom scores of 108 patients in placebo and 112 in test group were analysed statistically.

Symptom scores

The symptom scores of all available patients in both placebo and KalmCold™ groups were summarized in Table 2. There was no significant difference between the individual symptom scores of participants of KalmCold™ group as compared to placebo group on day 1. In both the groups, baseline (day 1) mean score of cough was the maximum while the score for sleep disturbance the minimum. In both the treatments, mean scores of all symptoms showed a decreasing trend from day 1 to day 3 but from day 3 to day 5 most of the symptoms in placebo treated group either remained unchanged (cough, headache and earache) or got aggravated (sore throat and sleep disturbance) whereas in KalmCold™ treated group all symptoms showed a decreasing trend.

Comparing mean within groups, mean scores of symptoms in both the groups decreased significantly ($p \leq 0.05$) from day 1 to day 3 and day 5 while from day 3 to day 5 all symptoms except expectoration in placebo group did not improve significantly whereas in KalmCold™ treated group all symptoms improved

significantly ($p \leq 0.05$) except earache. Comparing mean between both groups, the effect of both the treatments on all symptoms at day 3 was found to be the same i.e. treatments did not differ significantly while at day 5, all symptoms except earache in KalmCold™ treated group improved significantly ($p \leq 0.05$) than placebo group (Table 2).

Similarly, within groups, overall scores of all symptoms in both the groups decreased significantly ($p \leq 0.05$) from day 1 to day 3 and day 5 while from day 3 to day 5 placebo group did not improve significantly whereas KalmCold™ treated group showed significant improvement ($p \leq 0.05$). On between groups analysis, KalmCold™ group showed significant reduction ($p \leq 0.05$) in overall symptom scores as compared to placebo group (Table 2).

The effect (i.e. mean difference from baseline to final assessment) of placebo and KalmCold™ treatment on individual symptoms of common cold was presented in Table 2. The comparison showed that the effect of KalmCold™ over placebo was significant ($p \leq 0.05$) for all parameters except earache. The overall effect size (i.e. mean difference of effect between two groups) of KalmCold™ ($d=81.97$, $SE=10.09$, $t=8.13$, $p \leq 0.05$; 95% CI: 62.09 – 101.85) on symptom scores was found to be significantly different ($p \leq 0.05$) and 2.1 (52.7%) times higher than placebo.

Table 1
Summary of demographic information

Variables	Placebo	KalmCold™	t-value
Patients (M/F)	68 / 42	75 / 37	-
Age (yr)	32.42 ± 1.10	34.36 ± 0.97	1.33 ^{ns}
Weight (kg)	56.52 ± 1.00	56.23 ± 0.81	0.22 ^{ns}
Height (cm)	162.96 ± 0.66	162.46 ± 0.65	0.55 ^{ns}
Heart rate (min)	81.21 ± 0.72	80.94 ± 0.76	0.26 ^{ns}
BP diastolic (mmHg)	124.55 ± 1.07	125.09 ± 0.94	0.38 ^{ns}
BP systolic (mmHg)	79.23 ± 0.58	79.16 ± 0.58	0.08 ^{ns}

Values are expressed as mean ± SEM; n=110 in placebo and n=112 in KalmCold™

^{ns} – Non significant.

Symptom severity

The overall symptoms aggravated or unchanged in patients of two treatment groups were summarized in Table 3. In placebo group (out of 108 patients), symptoms of fourteen patients got aggravated (i.e. increased at the end of final evaluation as compared to baseline) while one in KalmCold™ group (out of 112). The proportions of patients whose symptoms got aggravated was found to be significantly ($Z = 3.28$; $p \leq 0.05$)

Table 2
Effect of KalmCold™ on symptoms of common cold evaluated using VAS.

Symptoms	Treatments	Day 1		Day 3		Day 5		Effect ^x	Effect size ^y (KalmCold™- Placebo)	
		n	Mean scores	n	Mean scores	n	Mean scores			
Cough	Placebo	89	55.06 ± 1.55	91	36.65 ± 1.66*	99	35.91 ± 1.60*	89	19.16 ± 1.70	15.45
	KalmCold™	89	57.87 ± 1.50	89	39.16 ± 1.64*	89	23.26 ± 1.81* ^{#,⊗}	89	34.61 ± 1.64 [⊗]	
Expectoration	Placebo	59	44.15 ± 1.60	59	32.03 ± 1.68*	65	29.85 ± 1.44* ^{#,⊗}	57	16.58 ± 1.77	12.61
	KalmCold™	65	47.08 ± 1.95	67	31.42 ± 1.94*	63	17.14 ± 2.32* ^{#,⊗}	62	29.19 ± 2.19 [⊗]	
Nasal discharge	Placebo	79	44.87 ± 1.84	78	29.49 ± 1.79*	82	26.65 ± 1.53*	76	18.95 ± 2.33	13.52
	KalmCold™	70	42.79 ± 2.14	71	25.70 ± 1.84*	70	11.50 ± 1.93* ^{#,⊗}	69	32.46 ± 2.16 [⊗]	
Headache	Placebo	62	40.08 ± 2.28	67	26.49 ± 2.03*	68	26.99 ± 1.80*	57	16.14 ± 2.22	12.11
	KalmCold™	64	40.86 ± 2.50	64	23.75 ± 2.49*	61	11.56 ± 1.97* ^{#,⊗}	60	28.25 ± 2.10 [⊗]	
Fever	Placebo	87	43.22 ± 1.97	86	27.03 ± 1.98*	80	23.88 ± 1.66*	76	21.32 ± 2.30	14.32
	KalmCold™	92	46.36 ± 1.93	91	25.22 ± 2.00*	87	11.03 ± 1.73* ^{#,⊗}	86	35.64 ± 1.72 [⊗]	
Sore throat	Placebo	70	46.57 ± 2.28	72	30.35 ± 2.31*	74	33.99 ± 2.04*	66	14.17 ± 1.84	17.73
	KalmCold™	82	46.46 ± 1.81	83	27.77 ± 2.07*	82	15.00 ± 1.88* ^{#,⊗}	79	31.90 ± 1.42 [⊗]	
Earache	Placebo	9	32.22 ± 5.21	7	18.57 ± 4.59*	7	17.14 ± 7.47*	6	18.33 ± 7.03	21.67
	KalmCold™	4	42.50 ± 16.52	4	22.50 ± 10.31 ^{ns}	5	6.00 ± 4.00 ^{ns}	4	40.00 ± 14.14	
Malaise/ Fatigue	Placebo	52	39.52 ± 2.67	51	28.04 ± 2.75*	47	24.26 ± 2.30*	40	18.88 ± 3.48	15.53
	KalmCold™	53	44.81 ± 2.88	53	23.87 ± 2.58*	52	10.29 ± 1.64* ^{#,⊗}	50	34.40 ± 2.72 [⊗]	
Sleep disturbance	Placebo	16	28.13 ± 2.28	15	14.67 ± 1.33*	13	16.15 ± 3.31*	13	13.85 ± 4.46	12.31
	KalmCold™	16	27.50 ± 2.14	17	12.35 ± 1.61*	15	3.33 ± 2.11* ^{#,⊗}	13	26.15 ± 2.90 [⊗]	
Overall	Placebo	108	216.20 ± 7.21	108	144.35 ± 5.91*	108	142.69 ± 6.34*	108	73.52 ± 6.98	81.97
	KalmCold™	112	222.14 ± 6.99	112	134.82 ± 5.76*	112	66.65 ± 5.60* ^{#,⊗}	112	155.49 ± 7.26 [⊗]	

Symptom scores are expressed as mean ± SEM.

* $p \leq 0.05$ Symptom scores of day 1 Vs Symptom scores of day 3 and day 5.

$p \leq 0.05$ Symptom scores of day 3 Vs Symptom scores of day 5.

⊗ $p \leq 0.05$ Placebo Vs KalmCold™.

^{ns} – Non significant.

^x Effect – Difference in mean scores between day 1 and day 5.

^y Effect size – Difference in mean effect between placebo and KalmCold™.

Table 3
Summary of symptom severity observed in patients.

Symptom severity	Placebo	KalmCold™	Z-value
Aggravated	14 (13.0%)	1 (0.9%)	3.28*
Unchanged	3 (2.8%)	1 (0.9%)	0.54 ^{ns}
Total	17 (15.7%)	2 (1.8%)	3.44*

Percentage of patients experienced the symptom severity is presented in parenthesis.

* $p \leq 0.05$ Placebo Vs KalmCold™; $n=108$ in placebo and $n=112$ in KalmCold™.

^{ns} – Non significant.

higher in placebo group than KalmCold™ group. The symptoms of three patients in placebo group and one in KalmCold™ group remained unchanged (i.e. same at baseline and at the end of the treatment) and the difference in proportions of symptoms unchanged between two groups were found to be the same ($Z=0.54$, $p > 0.05$). Overall, a total of seventeen patients in placebo group and two patients in KalmCold™ group did not show response to the treatments and the difference in the proportions was found to be significantly higher in placebo group than KalmCold™ group ($Z=3.44$, $p \leq 0.05$).

Adverse effects

The KalmCold™ group had total of six patients suffering from minor adverse effects, one patient each with vomiting, epistaxis, urticaria and three with diarrhoea. Of the three with diarrhoea, in addition one each had nausea or lethargy. The placebo group had three patients with adverse effects, one each with diarrhoea, vomiting (both mild in severity) and moderate rigor. The adverse effects between two groups were found to be same ($Z=0.63$, $p > 0.05$). In eight patients the effects were mild and isolated, and in one patient the effect was moderate and isolated. Except for vomiting (patient in KalmCold™ group) and urticaria, all other effects stopped spontaneously without any medical aid.

Discussion

Upper respiratory tract infections, caused by viruses, are most common diseases of human beings and on an average, adult are reported to have two to five common colds each year and school children from seven to ten colds per year (Eccles, 2005). In the light of gaining popularity of herbal preparations as remedies for various ailments, safe and effective herbal products would be of immense help. *A. paniculata* has been used in traditional system of medicine for promoting healthy immune system (Puri et al., 1993). It is incorporated as a principal ingredient in a number of proprietary preparations (Mishra et al., 2007). It is also recommended for treatment of fever associated with infectious diseases in Chinese and Thai traditional medicine (Coon and Ernst, 2004).

In the present study, the effect of extract of *A. paniculata* (KalmCold™; 100 mg, twice daily for 5 days) in patients with uncomplicated URTI was evaluated using VAS as the changes in pre and post-treatment scores of symptoms viz., cough, expectoration, nasal discharge, headache, fever, sore throat, earache, malaise/fatigue and sleep disturbance. The efficacy assessment of KalmCold™ essentially involved improvements in self evaluated scores of symptoms as most treatments for URTI are symptomatic. Clinical studies on the effectiveness of new treatments generally focus on changes in individual symptom scores as the main parameter of efficacy than any alterations in viral load. Additionally, it is also imperative for clinical evaluations on novel treatments for URTI to reveal outcomes in terms of decrease in

intensity/duration of symptoms since these end points are the key benefits for patients in distress (Eccles, 2005). The results, as a whole, showed that reduction in symptom severity scores were indicative of effect of KalmCold™.

A cursory view at the intricate display of anti-inflammatory, immunostimulant and anti-pyretic properties exhibited by *A. paniculata* explains its effect on common cold. Cough, a common symptom associated with URTI is believed to be caused by inflammatory mediators on airway sensory nerve endings (Jacoby, 2004). Clinical studies on common cold included intensity/frequency of cough as one of the primary outcome measures and reported that *A. paniculata* extract alone or in combination with *Acanthopanax senticosus* (*A. senticosus*) was superior to placebo (Caceres et al., 1999; Melchior et al., 2000). The findings of the current study indicated that cough was graded with maximum VAS scores in both groups and KalmCold™ has shown progressive efficacy during the treatment period. Similar significant decrease in severity was noticed for expectoration also. Nasal secretion (rhinorrhoea), an early symptom of URTI that often accompanies sneezing, varies with the time course of infection and the severity of inflammatory response (Eccles, 2005). Significant reduction in severity scores of nasal discharge was observed after 5 days of treatment with KalmCold™ and was in accordance with the findings of Caceres et al. (1999) and Gabrielian et al. (2002).

A double blind study has shown that significant improvements from shivering (feverishness) was achieved in patients with common cold at day 4 of treatment with standardized extract of *A. paniculata* (Kan Jang; 1200 mg/day). In the current trial, similar reduction in fever scores was observed with KalmCold™ on day 5 (Hancke et al., 1995). Sore throat, in particular relevance to rhinoviral infections, is related with the formation of bradykinin in the upper airway in response to viral infection (Proud et al., 1988). As is evident from Table 2, sore throat was found to be significantly reduced by KalmCold™ treatment on day 5. Beneficial effects of *A. paniculata* in alleviating sore throat were demonstrated in previous studies (Thamlikitkul et al. 1991; Hancke et al., 1995; Gabrielian et al., 2002).

Cytokines released from immune cells can be considered as the common mediators of headache associated with infections (Smith, 1992). Treatment with KalmCold™ decreased the symptom scores of headache from 40.86 ± 2.50 (day 1) to 11.56 ± 1.97 (day 5). KalmCold™ also resulted in significant reduction ($p \leq 0.05$) in symptoms scores of malaise/fatigue than placebo group indicating the effectiveness of the treatment over the physical and psychological entities of common cold reportedly influenced by the cytokine cascade (Mahoney and Ball, 2002). Gabrielian et al. in 2002 found that general malaise was significantly improved in test group wherein participants took composition containing 85 mg standardized extract of *A. paniculata* and 10 mg of *A. senticosus* extract for 5 days. Likewise, sleep disturbance, a manifestation of sickness behaviour syndrome induced by the cytokines (Capuron and Miller, 2004) was also significantly decreased in the KalmCold™ group.

From Table 2, it is evident that no significant differences in mean base line scores of symptoms (day 1) between KalmCold™ and placebo group during commencement of the trial. However, given the fact that the overall symptoms scores reduced significantly in synchronization with the effect size on day 5 confirmed the benefits of KalmCold™. Contrarily, placebo group showed no further relief in outcome measures except for expectoration on day 5. The minimal incidence of worsening or persistence of the clinical condition during final evaluation observed in KalmCold™ group (1.8%) in contrast to the substantial proportion of the placebo group (15.7%) also authenticated the efficacy of KalmCold™. Additionally, our findings are

supported by the earlier reports of clinical studies that revealed significant improvements in patients with URTI (the overall effect) upon short term treatment with *A. paniculata* extract alone or with *A. senticosus* (Hancke et al., 1995; Caceres et al., 1999; Melchior et al., 2000; Gabrielian et al., 2002). Meta analyses of clinical trials also showed that *A. paniculata* extract alone or in combination with *A. senticosus* was more effective than placebo and can be an appropriate alternative for treatment of uncomplicated cases of URTI (Coon and Ernst, 2004; Poolsup et al., 2004).

As regards the safety aspects, treatment with KalmCold™ was found to be well tolerated in human subjects. The side effects observed in the present study were mild, spontaneously recovered and did not require any medical aid except for vomiting and urticaria. Similar events were also recorded in published literature (Thamlikitkul et al., 1991; Melchior et al., 1997; Calabrese et al., 2000; Coon and Ernst, 2004).

In conclusion, the findings showed that KalmCold™ treatment significantly decreased all the symptom scores except for earache whereas in the placebo group the symptoms were either unchanged or got aggravated after day 3 during the study period. In the present study, treatment with KalmCold™ for uncomplicated URTI revealed the potentials of extract of *A. paniculata* and was found to be 2.1 times or 52.7% more effective than the placebo.

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