The present investigation centres on the scientific validation of *Calotropis procera* (Ait.) R.Br. (Asclepiadaceae) for its anti-malarial efficacy. A successful, dispensable formulation is established with standardization of dose for the complete treatment of *P. falciparum* and *P. vivax* malaria. People of India used about 65 plants belonging to 38 families to treat malaria (Bora et al., 2007). Different plant parts such as leaf, root, bark, fruit and the whole herb were used for making the traditional preparations. In the present work apical bud, flower and latex were used for the treatment purpose. In some cases the ingredients of the herbal preparation also included honey or sugar. In the present case pepper and sugar were added in the formulation as yogvahi (yogvahi=which increases the potentiality and the bio-availability of medicine in the body).

1. **In vitro screening of extracts from plant parts for schizonticidal activity**

In present investigation apical bud, flower and latex of *Calotropis procera* (Ait.) R.Br. (Asclepiadaceae) were selected for schizonticidal screening. Ethanol extract of apical bud and flower were tested for schizonticidal activity by Sharma and Sharma (2000), but latex was not evaluated. The extracts tried in the present work were aqueous decoction and acetone extract, were also not evaluated in the earlier reported works. The extracts derived from latex were found to be more active than the apical buds and flowers. The similar potentiality of latex was also observed in *P. berghei* infected mice and in human trials. The latex used in the present formulation was equally effective to cure *P. falciparum* in three days, which is comparable to chloroquine (CQ) treatment. The extracts derived from the plant parts showed lesser *in vitro* schizonticidal activity compared to CQ. This may be because of the fact that the synergistic effect of different chemical species is more pronounced in body than the effect under *in vitro* conditions.
The compounds freely soluble in RPMI-1640 culture media could only inhibit parasites during *in vitro* testing. Synergistic effect of plant powders (latex, apical bud and flower) produced the best results in human subjects instead of *in vitro* and *in vivo* testing of extracts from the same plant parts.

Searching of new compounds from plants that have been used ethnobotanically is the major trend of discovery of new drugs to treat diseases. For malaria, five alkaloids were isolated from the stem bark of *Zanthoxylum tsihanimposa*, traditionally prescribed to treat malaria in the eastern region of Madagascar (Milijaona *et al.*, 2003). Three quassinoids isolated from the roots of *Eurycoma longifolia* Jack were evaluated *in vitro* for schizonticidal activity (Ang *et al.*, 1995). *Aralidium pinnatifidium*, *Androgarphis paniculata* and *Goniothalamus scortechinii* were studied based on their traditional efficacy in the treatment of malaria (Najila *et al.*, 2002). *In vitro* and *in vivo* studies of Malaysian medicinal plants named *Piper sarmentosum*, *Andrographis paniculata* and *Tinospora crispa* showed considerable anti-malarial effect (Najib *et al.*, 1999). The aqueous extracts of traditionally used 14 medicinal plants for malaria in South Africa were evaluated for schizonticidal activity out of them only 10 plants were found to have considerable activity (Nundkumar *et al.*, 2002). Different extracts from 11 West African plants used traditionally against malaria in Ghana were tested for schizonticidal activity. (Köhler *et al.*, 2002). From the promising results, *Microglossa pyrifolia* (Lam.) Kuntze (Asteraceae) was chosen for further investigation and 13 compounds were isolated. Out of that 1-Acetyl-6E-geranylgeraniol-19-oic acid and sinapyl diangelate are new natural compounds. The two diterpenes E-phytol (IC$_{50}$:8.5μM) and 6E-geranylgeraniol-19-oic acid (IC$_{50}$:12.9μM) were proved to be the most active constituents of *Microglossa pyrifolia*. *Sida acuta* Burm. (Malvaceae) from Ivory Coast was traditionally used for
malaria (Banzouzi et al., 2004). Water decoction of the powdered plant was used by traditional healers; therefore water decoction and ethanol extract were evaluated. The IC$_{50}$ values obtained for these extracts ranged from 3.9 to 5.4μg/ml. Purification of this active fraction led to the identification of cryptolepine as the active antiplasmodial constituent of the plant. In vitro antimalarial activity of isolated compounds namely anthothecol, gedunin, limonoids, limonin and obacunone from Khaya anthotheca (Meliaceae) was evaluated by Lee et al. (2007). Anthothecol has showed 0.17μM IC$_{50}$ value. Gedunin had 0.14μM IC$_{50}$ value. The citrus limonoids, limonin and obacunone did not show any antimalarial activity. Testing of in vitro antiplasmodial activity of 18 plants used by traditional healers in Congo Brazzaville for malaria was evaluated with sixty-six extracts. Ethanolic and dichloromethane extracts of 7 among the 18 studied plants were moderately active (Mbatchi et al., 2006). Serial extractions in hexane, dichloromethane, and methanol of 14 plants used traditionally for malaria from Peru were tested for in vitro antiplasmodial activity. Seven plants displayed antiplasmodial activity with IC$_{50}$ from 2 to 25μg/ml (Roumy et al., 2007). In order to evaluate the anti-malarial potentiality of traditional remedies, 35 remedies were prepared in their traditional form used in French Guiana, and were screened for blood schizonticidal activity in vitro on P. falciparum strain. Five remedies displayed an in vitro IC$_{50}$ values <10μg/ml. The plant parts used together with these multi-ingredient recipes were leaves and stem of Picrolemma pseudocoffea, Pseudoxandra cuspidata and Quassia amara. Some of these remedies were screened in vivo against Plasmodium yoelii rodent malaria. Four remedies, widely used among the population as prophylactics, were able to inhibit more than 50% of the parasite growth in vivo at around 100 mg/kg. These remedies contain mainly Irlbachia alata (Gentianaceae), Picrolemma pseudocoffea (Simaroubaceae), Quassia amara (Simaroubaceae), Tinospora crispa (Menispermaceae) and Zanthoxylum rhoifolium.
(Rutaceae) as ingredients (Bertani et al., 2005). The same kind of *in vitro* and *in vivo* testing was performed for water decoction; acetone and methanol extract of *C. procera* plant parts in the present investigation. The IC\textsubscript{50} values obtained were in the range of 0.12 to 1.6 mg/ml. The most active extracts were evaluated for *in vivo* testing on *P. berghei* infected mice. The extracts have shown considerable anti-malarial activity and the major constituents of active extracts have been identified.

Most of the traditional preparations are orally administered either as a crushed extract or juice, leaf infusion or decoction. Water decoction is the most preferable medium for traditional drug administration. The hard parts such as root and bark are mostly preferred to be taken in the form of decoction. Out of 65 reported anti-malarial plants of India, 21 were used in the form of decoction. Therefore, in the present investigation water decoction was mainly validated for *in vitro* and *in vivo* anti-malarial activity. The water decoction of latex was most active among all tested extracts. The probable reason could be the aqueous nature of the latex, having water soluble secondary metabolites in higher concentration than any other parts of the plant. Among the three extracts tried water decoction was active in the case of latex, otherwise for other plant parts (apical bud and flower) acetone was the most active, methanol moderately active and water decoction was least active. Normally water and ethanol extracts are used by traditional healers because none of the other solvents are available with them. Therefore water and alcohol extracts were selected for basic screening of plant parts. In general, it was noticed that organic extracts were more active than the water extracts, with some exceptions. For example 18 plants from Ivory Coast used by traditional healers for the treatment of malaria were evaluated by *in vitro* anti-malarial testing. The powdered plants were extracted by water decoction, ethanol (95%) and pentane. The evaluated IC\textsubscript{50} value ranged between 18 µg/ml
to more than 500 μg/ml for aqueous and ethanol extracts and from 4.3 μg/ml to more than 500 μg/ml for pentane extracts (Ménan et al., 2006).

Plants commonly used in traditional medicine are safe due to their long usage in the treatment of diseases according to knowledge accumulated over centuries. For example herbal remedies are very common in the rural areas of Western Kenya (Sub-Saharan Africa). Due to sharing of traditional knowledge majority of population prescribed herbal medication at home rather than visiting health centers. However increased attention should be paid to the role of home treatment of malaria when policies are being developed for the management of febrile illnesses (Ruebush et al., 1995). In that light the herbal remedy was established in the present investigation to treat malaria victims without any side effect and toxicity with success ratio as good as conventional anti-malarial therapies.

2. In vivo extract testing against *P. berghei* infected mice with toxicity study

The plant parts used in medicine preparation in this work were latex and apical bud. Therefore, water decoction of both plant materials were evaluated on mice model for *P. berghei* infected malaria. The effect of latex decoction in comparison to CQ has been similar, both cured malaria in to the same duration in C57 mice infected with cerebral staged malaria. In comparison of *in vitro* testing, the efficacy of extract was more than that in mice model with CQ comparison. This is due to the physiological support given to the body for combating malaria parasite through the compounds present in extract besides active molecules. The decoction of apical bud was less effective compared to latex, therefore it was tested on normal blood staged malarial in Swiss White mice. Treatment
was found to reduce the level of parasitemia in comparison to the control but not achieved the effect as good as CQ. Mishra et al. (1991) reported that the ethanol extract of aerial part of *C. procera* excluding roots has shown no effect on NK 65 mice infected with *P. berghei*. This supports our approach to use apical buds and latex in the formulation being advantageous instead of entire plant.

The effect of herbal drug was more when it targeted parasite through physiological mechanism of the body instead of direct *in vitro* action on parasite. The activity of tablets and capsules prepared from latex and apical buds in human victims in the present investigation was more commendable as compared to mice model. This may be due to the fact that the drug does not perhaps interact directly with parasite as antiplasmodial drug but in association with human physiology the end result is promising. The present investigation does not consider the study on animal models as an authentic replica of human trials for all essentialities. The human physiology was found to be effectively synergistic associated with the drug to eradicate parasitemia. Human trials were more promising for validation of Ayurvedic formulations.

The level of toxicity studied for latex and apical bud on Swiss albino mice in the present study has shown no severe side effect or mortality. It has proved entirely safe drug to be used for human consumption. This plant is alleged as a poisonous plant but from the present investigation and reported toxicity study, it is proved harmless for consumption. The studies reported that sheep and cattle feed with fresh leaf and flowers of *C. procera* at 5 g/kg bodyweight for 30 days and 10 g/kg for another 30 days had not shown any clinical or gross pathological sign of poisoning and no histological sign in liver or kidneys except weight loss in these animals (Radunz et al., 1983; 1984).
The traditional parasitic remedy used in the Middle East was *Nigella sativa* (black seed) which was tested for its anti-malarial activity on *P. berghei* infected mice. The ethanol extract of seeds (100μl/kg) suppressed 70.59% parasitemia, chloroform extract (100μl/kg) suppressed 68.24% parasitemia and aqueous extract (400μl/kg) suppressed 69.39% parasitemia with intraperitoneal injection (Abdulelah and Zainal-Abidin, 2007). Applied extracts have only suppressed parasitemia and increased survival time of the mice; they did not cure mice completely as CQ. The same was observed for the ethanol seed extract of *Picralima nitida* (Nigeria) which was evaluated in *Plasmodium berghei* infected mice. It has increased mean survival time but not cleared parasitemia as that of CQ (Okokon et al., 2007). Even the isolated molecules like *Isosungucine*, an alkaloid isolated from the roots of *Strychnos icaja*, has shown antimalarial activity against the *P. vinckei petteri* murine infected mice by suppression of only 50% parasitemia with the dose of 30 mg/kg by intraperitoneal route (Philippe et al., 2007). In comparison, aqueous decoction of latex from *C. procera* has suppressed 100% parasitemia and cured mice from the cerebral staged malaria in the same duration as CQ in the present investigation, with the dose of 500mg/70kg (7.14mg/kg) by oral route.

*Azadirachta indica* is famous for its anti-malarial and anti-viral activities. The tablets prepared individually from the bark and leaf of *Azadirachta indica* were evaluated on *Plasmodium yoelli nigeriensis* infected mice by Isah et al. (2003). The tablet suspensions exhibited high prophylactic, moderate suppressive and a very minimal curative schizonticidal effect. No animal was cured of the infection in the curative test and there was not much increase in the survival time of the animals compared to the control. The suspensions of individual tablet from the leaf and bark reduced parasitaemia up to 79.6% and 68.2% respectively at the dose of 800 mg/kg. The calculation from the results showed
that an adult human would need to ingest a minimum of 48 g of the powdered plant material per day, an amount that is impracticable (Isah et al., 2003). The tablets prepared from latex of *C. procera* in the present work cured victims in total dose of only 1050mg within 3 days which is at par with CQ.

3. Chemical profile of active extracts

Latex is a colloidal secretion from the laticiferous tissue of the plant. Latex of *Calotropis procera* is well known for cardiac glycosides and hydrocarbons. The reported cardiac glycosides have been Calotropogenin (Haslam, 1996), Calotropin (Tyler, 1999), Uscharin, Calotoxin and Calactin (Seiber et al., 1982) with identification of some hydrocarbon derivatives like Linoleic acid, Oleic acid and Palmitic acid (Duke, 2007). More than fifty phytochemicals have been reported from *Calotropis spp.* (Duke, 1992A). The compounds investigated in the present work are different from the reported compounds.

The plant samples investigated for phytochemical analyses were apical bud and latex for their acetone and water extracts. Acetone extracts group of semi polar substances which evaporate at high temperature (<300°C), and separated through GC-MS for identification. The water extract contains polar compounds and mostly could not be evaporated at high temperature; therefore the active ethyl acetate fraction of it was analyzed through HPLC analysis. The spectra of GC-MS and HPLC were compared with available standard compounds reported from *C. procera* and the unidentified peaks of GC-MS were analyzed for the compounds which have not yet been reported from this plant. Collectively thirteen new compounds could be identified from acetone extract of latex
and four reported compounds from apical bud in the present work. The ethyl acetate fraction derived from water decoction of latex has shown major three compounds in HPLC spectrum.

4. Pharmacognosy of the plant parts used in herbal formulation

Pharmacognosy was carried out for dried apical buds and latex powder of *C. procera*, for HPTLC, IR, NMR, GC and XRF fingerprinting, with heavy metal detection, extractable values, ash value and growth rate of apical bud were examined.

HPTLC fingerprinting is the easiest and fast tool to identify raw powder sample from its characteristic pattern of separated phytochemicals on TLC plate. HPTLC fingerprinting was reported for roots and flowers of *C. gigantea* for petroleum ether (60-80°C) extract (Gupta *et al.*, 2005). HPTLC fingerprinting of latex and apical buds of *C. procera* has not been reported earlier, and therefore evaluated in the present work. HPTLC fingerprinting of apical buds showed higher number of spots than the latex sample because of pigments. In both the samples presence of β-sitosterol was confirmed. In latex profile, triolein and oleic acid were also confirmed in addition to β-sitosterol.

IR spectroscopy can be used in phytochemical studies as a ‘fingerprinting’ device (Harborne, 1998). Characterization of waxy hydrocarbons extracted from *Pedilanthus tithymaloides* in petroleum ether (b.p. 60-80°C) was characterized with the fingerprinting of Infra-red bands (De and Mukherji, 1997). Such type of IR fingerprinting was obtained for latex samples of *C. procera* in this investigation. The latex samples of different species, seasons and geographical locations were screened by IR spectroscopy to find the
level of variation occurred in different batches of tablets. The prepared tablets were also evaluated by IR spectroscopy to find out changes during preparation. The results indicated presence of major seven organic groups of compounds in latex and no difference could be recognized in the samples collected from two different species, three seasons and two places. Prepared tablets showed no spectral change except addition of binding and filling materials. In addition to IR, the spectra of NMR from latex confirmed the presence of three organic groups in support of the findings derived from IR spectra.

The GC characterization was obtained for essential oil of \textit{C. procera} latex. The total oil percentage from the seeds of \textit{Calotropis gigantea} was reported 30.8\% (Rao \textit{et al.}, 1983), but the level of essential oil from latex was not reported. The essential oil is responsible for the odor of latex. Latex of \textit{C. procera} was investigated for mainly hydrocarbons as renewable sources of energy (Kalita and Saikia, 2004), but none of the references have been obtained for characterization of compounds which are responsible for odor of the latex. This has been worked out in the present work. The latex collected in winter has the maximum intense odor and the amount of essential oil obtained was maximum from winter collection. The intensity of odor was less from summer collection and almost nil in monsoon collection. The amount of essential oil obtained from seasonal collection was in the proportion of intensity of odor. The magnitude of odor intensity and amount of essential oil helps to identify the seasonal difference in latex samples. The GC spectra of essential oil of latex also served characteristic identification for the sample. Seven standard compounds were compared with separated peaks that can be used for confirmation of the sample.
XRF analysis of latex and apical buds gave the elemental comparison of both the samples. It provided qualitative and quantitative data for the elements present in plant samples. Such type of comparative elemental analysis has not been reported in literature for *C. procera*. It gives the idea of seasonal variation of the availability of elements and its comparison with the soil that give the idea of elements taken up by plant. The results provided comprehensive idea of the comparison with major structural elements (C & O) to the trace elements required in physiological processes (Na, K, Ca, etc.). The reported major elements from the hexane extract of whole plant were 78.03%, 11.22%, and 10.71% C, H, and O respectively. The subsequent methanol extract from whole-plant residues after hexane extraction had 40.88%, 6.86%, and 30.05% C, H, and O respectively (Erdman and Erdman, 1981). This indicates that hexane extracts the compounds that have high number of carbon atoms but low number of oxygen atoms. In the present study value of carbon and oxygen investigated through XRF from the entire apical bud were 64% and 34% respectively. The amount of oxygen from apical bud was lower than hexane extract and higher than methanol extract of whole plant and the oxygen level in apical bud powder was higher from both the extracts of whole plant than that reported earlier. The carbon and oxygen level investigated from latex were 63% and 36% respectively. Latex of *C. procera* can be used as a renewable source of hydrocarbon fuels and intermediate energy resources.

Detection of heavy and toxic metals in prepared formulations is very essential before being administered to the patients. The heavy metals investigated were lead (Pb), cadmium (Cd) and mercury (Hg) with toxic metal arsenic (As). Presence of all detected metals in the tablets as well as capsules was below the permissible limit recommended by FDA, and therefore could be recommended for the consumption of human.
Extractable values of latex and apical bud were evaluated to judge the amount of soluble material extracted in different solvents. The reported extract values in literature were for root and flower of *C. gigantea* only for ethanol and water extracts (Gupta *et al.*, 2005). Reported extracts values for hexane-Soxhlet extractions of oven-dried whole plants, stems, leaves and pods of *C. procera* yielded 4.35, 3.83, 5.13, and 9.37 weight % respectively and subsequent methanol-Soxhlet extractions of residues previously extracted with hexane yielded 16.14, 18.50, 12.15, and 20.98 weight % respectively (Erdman and Erdman, 1981). Behera *et al.* (1995) analyzed hexane and methanol extracts of stem and leaves of *C. procera*. Erdman and Erdman (1981) studied extract values for apical bud in petroleum ether and ethanol. Arya and Kumar (2005) reported 25% yield from dry latex of *C. procera* in petroleum ether extract followed by methanol extract. In the present work extract value of latex is 38.4% in the acetone extract followed by chloroform extract and ethanol extract.

The reported total ash for root is 8%, out of that acid-insoluble ash is 3.5% and total ash for flowers is 10%, out of that acid-insoluble ash is 1% for *C. gigantea* (Gupta *et al.*, 2005). The reported findings are different than the investigated ash values of apical bud and mature leaf of *C. procera*. The amount of ash content reported for the whole plant of *C. procera* was 11.054 % (Erdman and Erdman, 1981). The ash values for subsequent hexane and methanol soxhlet dried extracts were also reported for *C. procera* whole plant as 0.71% and 12.02% respectively, the results indicate good amount of extraction in methanol (Erdman and Erdman, 1981). The plant part used in formulation was apical bud. Therefore ash content was specifically evaluated for it and compared with the ash value
of mature leaves. The investigated ash values of apical bud and mature leaves compared with reported ash value of whole plant have not shown much difference with each other.

The growth rate study is important if cultivation is required in future. There are no agronomic data available for this plant. The harvesting schedule has been studied for apical buds from the field trials conducted in the present investigation.

5. Clinical trial of herbal regimen

Clinical trials of herbal therapeutics are the scientific validation of traditionally used treatments. More than seventy cases of clinical assessments of herbal therapeutics for major human disorders are compiled together in the form of book by Kumar (2007). The report of clinical trial on malaria for herbal components available for Ayush-64, comprised four medicinal plants namely \textit{Picrorhiza kurroa} (Katuka), \textit{Alstonia scholaris} (Saptachada), \textit{Swertia chirata} (Kirataittaka) and \textit{Caesalpinia bonducella} (Kuberakshi) (Anonymous, 1987). The response of Ayush-64 in double blind clinical study against \textit{P. vivax} was 72.4\%, with the dose of 3g/day for 4 days (12g/treatment). Patients were kept in hospital during treatment. The similar line of out door clinical trial on human subjects was carried out in the present work for the herbal regimen prepared from \textit{C. procera}. The response noticed was 92.96\%. The dose required for treatment was 600mg/day for 7 days (total 4.2g/treatment with apical bud) for \textit{P. vivax} and total 1.05 g/treatment with latex for \textit{P. falciparum} treatment.

Clinical study was reported for herbal remedy comprising \textit{Gossypium arboreum}, \textit{Anacardium occidentale}, \textit{Citrus medica}, \textit{Phyllanthus amarus} and \textit{Lippia multiflora} as
main ingredients (Ajaiyeoba et al., 2004). The study was conducted in two different
groups one at Oyo (urban center) and another at Otu (rural center) in Nigeria. The
treatment with the herbal remedies cured 88% (23/29) *P. falciparum* infection in Oyo and
42% (13/25) in Otu. The results indicate more response in parasite strain of urban area
than the rural area. Parasite densities ranged from 171 to 53,613 parasites/µl blood and 87
to 36,209 parasites/µl blood in patients from Oyo and Otu respectively. In present clinical
trial, the latex of *C. procera* cured *P. falciparum* infection with 87.88% response and *P.
vivax* with 100% response. The cases treated with *C. procera* apical bud were cured
56.76% of *P. falciparum* and 95.65% of *P. vivax* cases. The therapeutic potentiality was
higher in the latex formulation than the apical bud formulation. Variable response against
parasite strain of urban and rural area was also observed in the present study. The failed
victims of *P. falciparum* and *P. vivax* both were enrolled from the village Chunel (Kheda
district) where CQ resistance cases were also present. It is the place 5 km away from
Surashamal where several deaths were reported due to malaria. Conclusively the
proposed herbal regimen has 100% success on malaria in urban area where CQ resistant
cases were also present. The density of *P. falciparum* parasitemia was in the range of
2514 to 63,636 parasites/µl blood into the treated victims which was higher than the
above mentioned study of Ajaiyeoba et al. (2004). The parasite clearance time was 48hrs
for *P. falciparum* with latex treatment and the same for *P. vivax* with apical bud treatment
in our clinical trials. The fever reduction time varied between 2-24 hrs for *P. vivax* and
12-48 hrs for *P. falciparum* infection treated with herbal formulations. These results are
comparable with those reported for CQ and other single molecule anti-malarial treatments
(Zongo et al., 2007, Ménard et al., 2007, Kamya et al., 2007). The additional advantage
of the treatment is, that it cures *P. falciparum* only in three days regimen without any
severe side effect and the recovery from the weakness becomes very fast instead of CQ treated patients. Moreover it has a long shelf life of about 36 months.

Chloroquine (CQ) remains the first-line therapy for uncomplicated malaria. The difficulties in treating drug-resistant falciparum malaria are compounded by the necessity of giving anti-malarials over long periods of time. The resultant decrease in patient compliance not only lowers cure rates but also predisposes to the further spread of drug resistance. A clinical trial of standard CQ therapy for uncomplicated malaria at the 28-day cumulative incidence of therapeutic failure was 95% for *P. falciparum*, 84% for *P. vivax*, and 100% for mixed infections. Only one subject each for *P. falciparum* and *P. vivax* remained free of parasites at day 28. These findings document almost complete failure of CQ against *P. falciparum* or *P. vivax* near the northeastern coast of Indonesian Papua (Sumawinata *et al.*, 2003). Looareesuwan *et al.* (1996) showed increased level of malarial resistance in artesunate plus mefloquine drug treatment. Sulfadoxine-pyrimethamine (SP) is often used after CQ treatment failure and has replaced CQ as the first-line treatment in parts of Africa. Compared to SP, significantly more patients treated with CQ developed early or late clinical failure. Consideration has been given to replacing CQ as the first-line therapy for uncomplicated malaria in Uganda, particularly in young children (Kamya *et al.*, 2001). Reports indicate a high risk of therapeutic failure of CQ against *P. falciparum* and resistance to CQ in *P. vivax* in Guyana (Baird *et al.*, 2002). In the studied effect of herbal treatment in the present work none of the adverse effects reported earlier were noticed on day 4. The victims treated with herbal regimen completely recovered after three days for routine work without feeling any weakness. *Plasmodium falciparum* resistance has rendered CQ monotherapy ineffective in much of Africa and data on alternative regimens are limited. Staedke *et al.* (2007) compared CQ+sulfadoxine-
Discussion
pyrimethamine, amodiaquine+sulfadoxine-pyrimethamine and amodiaquine+artesunate for treatment of uncomplicated malaria in Kampala, Uganda. In allopathic treatment multidrug therapy is the only solution for resistant malaria, but that makes the victim very weak and need long time for complete recovery. In such high risk area herbal drug can provide the best supportive medicine to treat resistant falciparum malaria. These major examples indicate the increasing resistance level against the routine malaria treatment. Moreover the risk of recurrent parasitemia is common in allopathic treatment with combination regimens of dihydroartemisinin-mefloquine (Na-Bangchang et al., 1999) and dihydroartemisinin-piperaquine (Kamya et al., 2007). Chances of resistance development against herbal treatment is very less, because it acts synergistically and therefore development of resistance even after a long period of time is not observed as in the case of single molecule regimen. Therapy with single molecules makes the parasite resistance soon for the drug. Use of such herbal treatment at routine bases in OPDs and by private practitioners prevent parasite to become resistant soon. When such remedy failed in extreme endemic rural areas than the allopathic treatment should be switched on. This policy could reduce the rate of occurrence of parasite resistance and the level of side effects of modern drugs in to the society. The prominent results of tested herbal treatment showed very less recurrence (3.02%) of \textit{P. falciparum} and prevents relapse for one year in \textit{P. vivax} by providing long time immunity against malaria.

6. Commercial production of medicine with quality control standards

The medicine was manufactured at PMAM Pharmacy, Nadiad maintaining GMP (Good Manufacturing Practices) standards during the research programme. It includes collection standards and processing of raw material used for medicine purpose. The criteria for
collection were established to harvest effective size of apical bud and good latex. During collection and processing of raw material hygiene was critically maintained. The fresh yield of apical bud/flowers was calculated. This gives the idea of required collection of harvest needed for apical bud and flowers from it. The dried yield was calculated to quantify the final yield. The medication formula was standardized for final production. The statistical data of production from each processed batch were noted. Costing for the prepared medicine was calculated and the final customer price per treatment was estimated. The price of medicine is economic and affordable for even poor people. Moreover the physical standards of raw material and the finest prepared medicine both were studied and the range was evaluated for the collection of different seasons. Microbial load was evaluated before the medicines were used in treatment. The data confirmed their dispensing successfully.

7. Antibacterial testing of plant extracts

*Kawath* - The water decoction of *Calotropis procera* was reported for wound dressing in Ayurveda (Charaka, 1965; Sharangdhar, 1955). Latex from *C. procera* is widely used in folk medicine as a rich source of biologically active compounds capable of promoting diverse benefits such as control of dermal infections and pain relief among other useful properties. The wound healing property of latex was evaluated on Guinea pigs by Rasik *et al.* (1999). In the present work anti-bacterial effect of solvent fractions derived from the latex and apical twig of *C. procera* has been studied. The bacterial strains selected were opportunistic wound pathogens and the bacteria responsible for typhoid infection.
Discussion

The basic screening of different parts of *C. procera* as antibiotic agent has been evaluated for leaves (Suresh and Chauhan, 1992), flowers (Larhsini et al., 2001), bark of root and stem (Jain et al., 1996). In the present work latex and apical twig were tested through *Kawath* preparation, and the results obtained prove antiseptic effect of ‘Kawath’ used for wound dressing in the traditional medication method. The results have indicated the effect of this plant against typhoid infection of *S. typhi* and *S. paratyphi*. The aqueous extract was further purified with solvent fractionations, out of that the most active ethyl acetate fraction was evaluated for MIC value. The obtained MIC value was in the range of 0.25 to 2 mg/ml. It has revalidated the antibacterial property of *C. procera* reported in Ayurvedic literature.

8. Micro-propagation of *Calotropis procera* from node explants

Direct organogenesis of *C. procera* has not been reported in the literature. This may be because of its occurrence as waste land weed. The waste lands are fast getting occupied by urbanization which results into habitat loss for this species. Moreover its occurrence in forest is almost nil because it can not grow as undergrowth. Therefore its availability in large quantity may become scarce in future. Therefore, *in vitro* propagation method from nodes of this plant has been established in the present work. The area explored in the field of tissue culture for *C. procera* was mainly for free cell culture to produce latex, which is still not successful for production at industrial level (Datta and De, 1986) because laticifer differentiation is less in free cell culture. *In vitro* plant regeneration was established through callus induction (Roy and De, 1990, Roy et al., 2000), but this method takes more time and labor than the direct organogenesis from ex-plant.
In the present work combinations of two auxins with two cytokinins were tried for direct shoot regeneration, out of that single combination of 2,4-D (0.5) + Kn (0.5) mg/l was selected for production of *in vitro* shoots.